Feeding, Growth, Respiration, and Carbon Utilization of a Euphausiid Crustacean[1]

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ABSTRACT

In the euphausiid shrimp *Euphausia pacifica*, a member of the oceanic plankton, growth was as rapid as 0.048 mm/day in juveniles in the laboratory, more than twice as fast as that observed for oceanic populations (0.02 mm/day) by other workers. Periods between molting, which occurs throughout the life span, varied between 3 and 8 days depending on the temperature but not on the amount of food eaten. Respiration accounted for the major portion of the assimilation of carbon (62–87%) and molting, growth, and reproduction accounted for the remainder. In mature animals, 9% of the assimilated carbon was released as the organic portion of the eggs. Rapidly growing animals incorporated as much as 30% of the assimilated carbon, but calculations for an oceanic population gave 9% as the incorporation of organic carbon into tissue (excluding eggs and molts) over the life span of the animal. Assimilation of ingested carbon (digestion) appeared to be high, usually over 80%, as judged from tracer experiments. In the laboratory, crustacean nauplii seemed to be a preferred food over unicellular algae but both were eaten when available; one alga was rejected. Nauplii must be smaller than 0.8 mm for many to be ingested by adult *E. pacifica*. Molting, which mainly occurred at night, depressed feeding. The filtering rates for the animal on unicellular algae are sufficient for growth and metabolism if the carbon available to the animal in the sea is equivalent to that contributed by both detritus and phytoplankton standing crop in the area of the northeastern Pacific Ocean where *E. pacifica* is found. It is suggested that adult *E. pacifica* must capture between 100 and 200 nauplii per day as 1-year-olds and 200–300 per day as 2-year-olds to satisfy all of its carbon requirements. The mean density of small crustaceans in nature is probably too low for this animal to fulfill its needs. However, concentrations may be high enough in local aggregations.

INTRODUCTION

This paper reports on an experimental study of the euphausiid crustacean *E. pacifica* and describes in quantitative terms its growth, molting, feeding, respiration, and the efficiency with which it incorporates carbon from its food into body tissue. The information obtained in the laboratory is compared wherever possible with observations made by others on the same species in nature.

Ultimately the marine scientist wishes to be able to predict the yield of fish flesh in the ocean through knowledge of primary production by the standing crop of phytoplankton. Without quantitative information on the efficiency with which organic carbon is transferred from one trophic level to another, no predictions of this kind are possible.

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Measurements of organic carbon production by phytoplankton are now made routinely in all the oceans of the world (U.S. Atomic Energy Comm., 1961), but few studies have been made on the utilization of this carbon by zooplankton. In recent years, following the pioneering work of Marshall and Orr (1955) on the copepod *Calanus finmarchicus*, some of the numerically more important planktonic animals, chiefly copepods, have been studied experimentally in the laboratory (see review by Conover, 1964).

Among the planktonic organisms in the food web of the oceans, the euphausiid crustaceans rank high in abundance and importance. They are food for a variety of fishes ranging from albacore (McHugh, 1952) to salmon (Merkel, 1957) and sardines (Hand and Berner, 1959) and are the chief food of baleen whales (Marr, 1962). Euphausiids may also contribute substantially to the detritus of the ocean because they molt regularly and frequently throughout their life (Lasker, 1964; Jerde and Lasker, 1966). An intimate knowledge of the physiology of euphausiids is essential before their potential and actual *in situ* ability to capture food and incorporate carbon can be known.

Euphausia pacifica Hansen, the most abundant euphausiid off the California coast, has been shown by Brinton (1962) to be an important indicator of oceanic water masses in the northern Pacific Ocean. Ponomareva (1954, 1955, 1959, 1963), among others, has investigated the ecology of this species and has analyzed information on feeding habits, growth, and fecundity from field collections. Osterberg et al. (1964) found this species to be an indicator of radioactivity contamination off the west coast of the United States and an important component of the food web of the northern Pacific Ocean. Boden et al. (1955) in an excellent monograph offer extensive discussion of the distribution of this and other euphausiids of the Pacific Ocean.

MATERIALS AND METHODS

Methods for the collection and maintenance of euphausiids in the laboratory were described by Lasker and Theilacker (1965). These techniques were used to capture and to maintain *E. pacifica* in the laboratory over 1 year at temperatures ranging from 6.5 to 16 C. *Euphausia pacifica* grows steadily and molts regularly on a diet of newly hatched *Artemia salina* nauplii. Euphausiids grow throughout their life and the uropods of the molts show successive increments in length which are proportional to the increase in "body length" which (Fig. 1) is in turn related to dry weight. "Body length" was measured as the length of the carapace from behind the eye to the junction of the abdomen and telson in straight animals. The relations between uropod length and body length (Fig. 2) allow the estimation of dry weight at any stage of growth by measurement of the uropod length at molting. Live animals were measured after being anesthetized in isotonic $MgCl_2$; after a rinse in distilled water, they were dried to a constant weight over silica gel in an oven at 50–60 C and weighed. Formalin-preserved animals could not be used because they have a different length:weight relationship; the weight for a

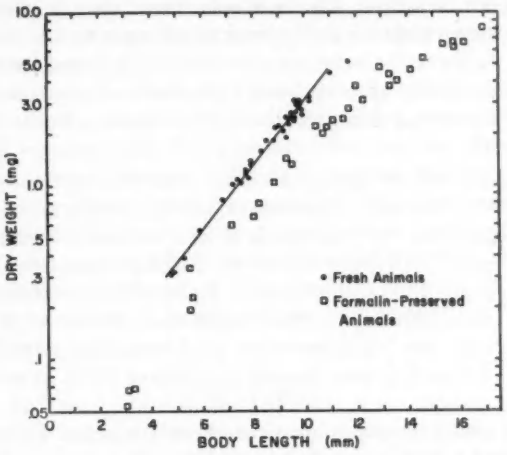

FIG. 1. Relation between body length and dry weight for fresh and formalin-preserved *E. pacifica*.

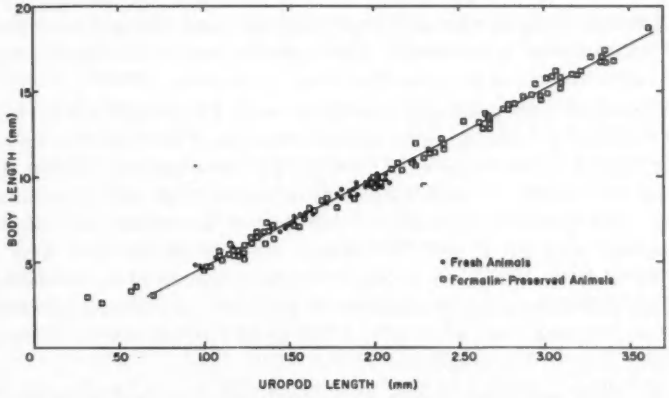

FIG. 2. Relation of body length (measured from behind the eye to the junction of the telson) to uropod length for fresh and formalin-preserved *E. pacifica*.

given length is considerably less than that of fresh animals (Fig. 1). Loss of body weight due to formalin preservation was further indicated by the low quantity of ash, 3.5% of dry weight, found in preserved euphausiids as compared to 10.5% of dry weight in fresh animals. The remainder of the loss is undoubtedly due to leaching of organic materials.

Each animal used in a growth experiment was observed daily, the temperature of the water was recorded, and molts were removed if present. The

molt uropods were measured, the molt was rinsed once in distilled water and dried on a microscope slide for subsequent weighing with a Cahn electrobalance to ± 0.002 mg. Some molts were preserved in 3% buffered formalin for uropod measurement only. Both uropods were examined in every case because occasionally one of them was damaged; when this occurred only the undamaged one was measured.

In experiments where carbon-14 was used, the technique was modified from Lasker (1960). In each experiment, 20–30 μc of carbon-14 were added as $NaHC^{14}O_3$ prepared by the method of Steemann-Nielsen (1952). The $NaHC^{14}O_3$ was inoculated into 60 ml of a dense suspension of a bacteria-free culture of a unicellular green alga, *Dunaliella primolecta*, *D. tertiolecta*, or *Platymonas subcordiformis*. After incubation under a fluorescent light for more than 24 hr, the algae were fed to *Artemia salina* nauplii which had just begun to feed. Feeding was allowed to continue 24 hr at room temperature after which the nauplii were collected on netting, rinsed in sea water, and transferred to a medium containing nonradioactive algae. Feeding on the nonradioactive algae for 20 hr caused the nauplii to clear their guts of undigested radioactive algae. At the temperature at which the experiments with the euphausiid were performed (ca. 10 C), *Artemia* nauplii respire and grow very slowly. For short-term experiments (of 1 day or less) the amount of carbon in the *Artemia* nauplii changed imperceptibly and the ratio of carbon-14 to total carbon was not altered. The calculations of carbon-incorporation efficiency were the same as those described by Lasker (1960).

All carbon-14 measurements were made with a Nuclear Chicago vibrating-reed electrometer (Dynacon) after wet combustion of the tissue to be analyzed to carbon dioxide (Van Slyke et al., 1951). This procedure obviated the problems usually encountered with Geiger counting such as self-absorption, back scattering, and variable geometry. Total carbon in animal and plant tissue was measured with an F and M carbon analyzer model 180. The method is based on a dry combustion at 800 C in the presence of an oxidizing agent (Ag_2O) and the subsequent separation of gaseous combustion products with a gas chromatograph. A lower limit of 100 μg of carbon can be detected with this apparatus to $\pm$ 1% precision.

Euphausiids and their molts were analyzed for total nitrogen by the standard Kjeldahl method. Ash was determined by combusting whole animals or molts at 500–525 C and weighing the residue.

Experiments on filtering rate were done by supplying *E. pacifica* with a known number of algal cells and by counting the cells remaining after a suitable interval of feeding time, usually 24 hr. Algal cells were counted with a Coulter Counter Model A. Calculations of milliliters cleared per unit time, or filtering rate (F), were made from the formula derived by Gauld (1951),

$$F = V \frac{(\log_{10} C_0 - \log_{10} C_t)}{(t \log_{10} e)}$$

where C_t is the concentration of cells at time t, C_0 the initial concentration, and V the volume. Corrections for increases in cell number due to cell divisions were made by reference to a control.

When *Artemia* nauplii were supplied as food, the actual number of nauplii was determined before and after the feeding to determine a filtering rate, or more properly, a capture rate. Experiments were done in the dark to assure the random distribution of nauplii in the experimental container.

Respiration was determined by standard Warburg manometry after acclimation of euphausiids to the testing temperature (usually 10 C) for more than 12 hr. In order not to disturb the animals excessively, the manometers were shaken 5 min each half hour at 60 cycles/min to establish gaseous equilibrium. Q_{O_2} (oxygen consumed) is reported here as microliters of O_2/milligram dry weight per hour.

GROWTH

Ecdysis is important in the physiology of euphausiids because growth, as in all arthropods, is accompanied by molting. Unlike copepods, however, euphausiids continue to grow throughout their life span. In an earlier paper Lasker (1964) observed that *E. pacifica* molts on the average every 5 days at temperatures similar to those at which the animal lives in the sea (ca. 9–15 C). Other euphausiid species from neritic as well as oceanic habitats are known to molt at about the same frequency in laboratory containers (Jerde and Lasker, 1966).

Temperature was found to be an important exogenous factor regulating molting in *E. pacifica*. For a large number of *E. pacifica* ranging from 1 to 9 mg dry weight at the daily temperatures of the refrigerated sea water supplied in the Scripps Institution of Oceanography's experimental aquarium, the euphausiid's intermolt periods varied as the water temperature fluctuated (Fig. 3). For a single organism the intermolt period was shortened rapidly by an artificially produced warm period (Fig. 4). Three days seemed to be the minimum intermolt period (maximum molting rate); temperatures above 12 C did not accelerate molting further. Over a $3\frac{1}{2}$ month period at elevated temperatures near 15 C, 12 *E. pacifica* shed a total of 83 molts. Of these molts, 6% were spaced at 5 days; 53% at 4 days; 37% at 3 days; 3% at 2 days, and 1% at 1 day.

Each cast skin represents loss of a sizable quantity of organic material by the animal. Lasker (1964) pointed out that the dry weight of each molt is approximately 10% of the dry weight of the *E. pacifica* which produced it. This figure is also close to the average molt weight for six other species of euphausiids reported by Jerde and Lasker (1966). Dry weight of the molt from a single animal may vary from 4 to 14% of the animal's dry weight. Molts contained approximately 46% ash, 17% organic carbon, and 2.5% organic nitrogen (Table I). No inorganic carbon was detectable, therefore, about 4% of a euphausiid's carbon and 2% of its nitrogen may be lost at each ecdysis.

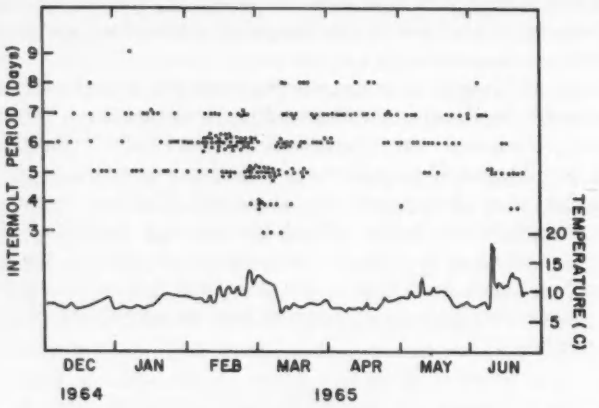

FIG. 3. Intermolt periods for individual *E. pacifica* in relation to the temperature of the laboratory sea water. Each dot represents one animal's intermolt period at the temperature indicated by the curve below. Additional animals caught in February 1965 account for the large number of dots in February and March.

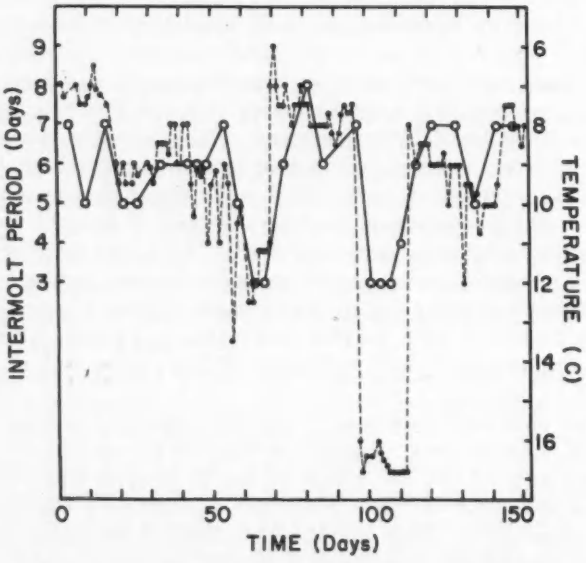

FIG. 4. Intermolt periods of a single *E. pacifica* in relation to temperature. To ascertain the effect of a change in temperature on molting, the sea water was intentionally warmed between the 97th and 112th days. Temperature is indicated by the broken line and closed dots; molting frequency is shown as open dots connected with a solid line.

TABLE I. Per cent chemical composition of *E. pacifica* and molts.

Item	*E. Pacifica* Mean	Range	Molts[a]
Water	82.8±3.8 (9)[b]	78.7–89.6	
Dry weight (by difference)	17.2		
Ash of dry weight	10.5 (5)	9.9–13.0	45.5
Carbon of dry weight	42.0±1.7 (22)	38.1–45.1	17.0
Nitrogen of dry weight	11.5 (5)	10.4–13.0	2.5

[a]Molts were pooled for analysis.
[b]Mean, standard deviation, and number of replicates.

INCREASE IN LENGTH

Growth in length as determined from measurements of molt uropods is illustrated in Fig. 5 and 6 for seven experimental animals supplied with a surplus of *Artemia* nauplii as food in 1-liter containers of sea water kept in the dark at 10 C. The three animals whose growth curves appear in Fig. 5 were observed soon after the furcilia stage of development as juveniles, i.e. each had developed a single terminal spine and had only two pairs of posterolateral spines as described by Boden (1950). These euphausiids showed a steady linear growth and almost equally spaced molts. In the smallest animals,

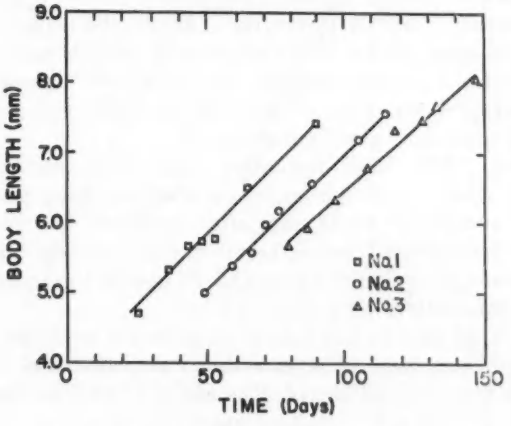

FIG. 5. Growth in length of three juvenile *E. pacifica* maintained in the laboratory at approximately 10 C. Gain in weight for these same animals is depicted in Fig. 7.

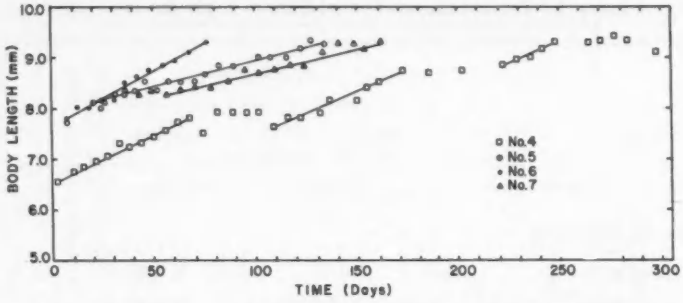

FIG. 6. Growth in length of four adult *E. pacifica* maintained in the laboratory at approximately 10 C. Gain in weight for these animals is depicted in Fig. 8.

No. 1 and 2 (Fig. 5), the lengths and dry weights were similar — 4.72 mm and 0.28 mg in animal No. 1, 4.95 mm and 0.30 mg in animal No. 2. Animal No. 3 was 5.65 mm and 0.50 mg at the beginning of the study of linear growth. The initial lengths of other animals in the growth experiments varied from 6.3 to about 8.0 mm. The smallest animals had the fastest growth rates, 0.034–0.048 mm/day.

The laboratory-maintained animals included euphausiids which did not grow in spite of sufficient food. Molting of these animals continued uninterrupted but the animals became progressively shorter and died within a few weeks or months. These examples indicate that molting is a metabolic necessity and continues at the expense of the organic reserves of the animal.

Following the rapid growth period of animals 1, 2, and 3 (Fig. 5) they were kept about 1 month between 10.5 and 13.5 C due to a failure of the laboratory seawater cooling system. The growth, after a short lag period, decreased drastically (to 0.0016 mm/day). When the water was cooled and maintained once again below 10 C, the former growth rate resumed, again after a lag period of 1 month. Feeding may have been adversely affected and the extra metabolic demand due to warming probably was enough to reduce the carbon that ordinarily went into growth.

Ponomareva (1959, 1963) has observed that *E. pacifica* spawns only once a year, in mid June, in the northwest Pacific–Bering Sea, and that the furcilia stage is reached in 16–18 days after spawning. She distinguished two modal groups characterized by different lengths[2] in her winter collections and deduced that the euphausiid grows to 10 mm in 1 year and to 15 mm in 2 years. Sexual maturity is achieved in 1 year.

The most rapid rate in laboratory experiments over the length interval of 5–8 mm (0.048 mm/day) was more than twice the rate (0.019 mm/day) calculated from the information of Ponomareva. The favorable conditions

[2]The telson of *E. pacifica* is 20% of the total length. To make the figures of Ponomareva (1963) comparable to the body length measurements reported in this paper I have subtracted 20% from the total lengths reported by her.

for the laboratory animals, particularly the availability of food, may account for the faster growth.

Increase in Weight

Animals 1, 2, and 3 had similar growth in weight (Fig. 7) as well as in length, and it may be inferred that all three animals were feeding to their maximum on the excess number of *Artemia nauplii* that were supplied.

Animal No. 1 increased in dry weight 0.0139 mg/day for 65 days at 10 C and shed 11 molts. The molts weighed less than the average for *E. pacifica*: 6% as opposed to 10–11% (Lasker, 1964; Jerde and Lasker, 1966) of the dry body weight at each molt. Animal No. 2 gained 0.0142 mg/day. In 67 days 12 molts were shed and each averaged 4% of the dry body weight. Animal No. 3 gained 0.0146 mg/day for 68 days with each of 13 molts averaging 4% of the dry body weight.

For the four other animals the increases in weight ranged from 0.004 to 0.015 mg/day (Fig. 8).

Increase in mass of individual *E. pacifica* over a period of 20 months after hatching was calculated for the Bering Sea population from Ponomareva's (1963) data. The calculations were not extended beyond this period because

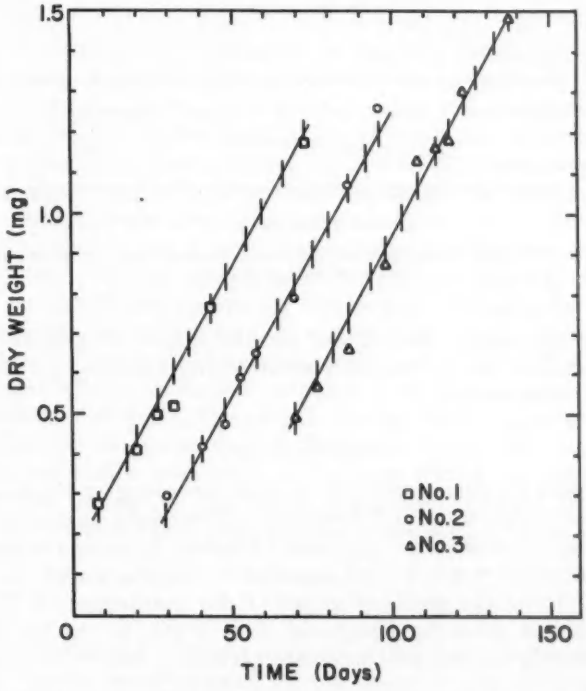

FIG. 7. Increase in dry weight of three juvenile *E. pacifica* maintained in the laboratory. Same animals as in Fig. 5. The vertical dashes indicate when molting occurred.

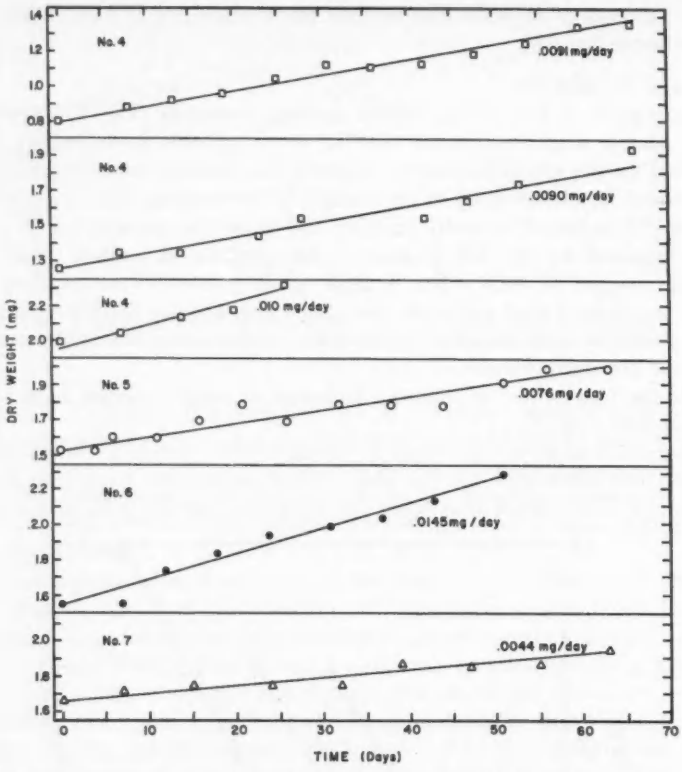

FIG. 8. Increase in weight of adult *E. pacifica* maintained in the laboratory. Same animals as in Fig. 6.

I lacked weight–length information for the larger individuals. *Euphausia pacifica* averaged 1 mg dry weight 7 months after hatching, 2.6 mg in 1 year, and 9 mg after 20 months.

RESPIRATION

The oxygen consumption of *E. pacifica* for young and old animals was determined. For animals with dry weights 1.05–9.65 mg held at 10 C (Fig. 9) the mean oxygen consumption (Q_{O_2}) was 1.47 μliter O_2 per mg dry weight per hr (standard deviation = ±0.35) and appeared to be independent of the size of the animal.[3] A vertical profile (Fig. 10) of the distribution of *E. pacifica* in the water column taken by Longhurst et al. (1966) in the San Diego area shows that during the daytime most are between 8 and 11 C. Clarke (1966)

[3]Euphausiids move their swimming appendages rapidly and continually, presumably to aerate their external gills. Oxygen uptake remains the same whether or not an animal is swimming.

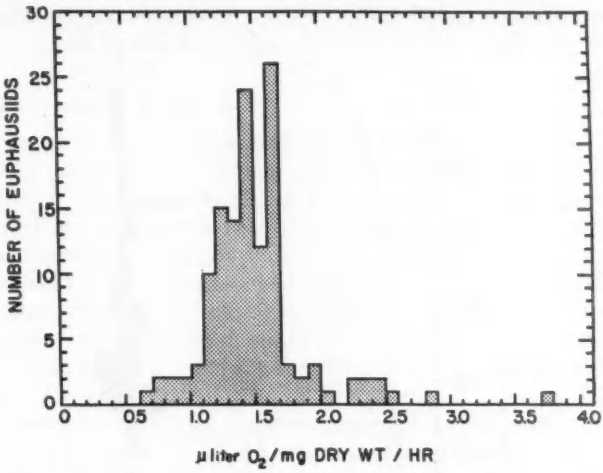

FIG. 9. Rate of oxygen consumption of 122 *E. pacifica* at 10 C. Dry weights ranged from 1.05 to 9.65 mg.

showed that this restricted distribution is due to a sharp self-confinement of the population to a specific light intensity (10^{-3} to 10^{-4} $\mu w/cm^2$) in the Santa Barbara Basin, which is northward of the San Diego Trough where I collected the euphausiids for this study. In 1965 Clarke (1966 and unpublished data) monitored the diurnal isolume situation for several days in each month, collected plankton at specific depths, and simultaneously recorded vertical temperature profiles. From the diurnal distribution of the isolumes I deduced the depth of the bulk of the *E. pacifica* population and the time spent at any specific temperature during representative days of each month. The detailed data will be published elsewhere by Dr Clarke and his associates[4] but he has kindly consented for me to use his data to calculate the average temperature for the *E. pacifica* population over the entire year. During daylight hours, regardless of the month, *E. pacifica* resides in 8–10 C water and rises rapidly into surface waters at dusk. The overall average temperature at which the bulk of the population resided during 1965 was 11.05 C. My measurements of euphausiid respiration were made at 10 C and I have not introduced any correction factor owing to the small difference between the test and environmental temperatures in comparison with the normal variation in oxygen consumption.

EFFICIENCY OF CARBON UTILIZATION

Carbon incorporation efficiency, as used in this paper, is the percentage of the ingested carbon which appears in the tissue of the euphausiid during

[4]General Motors Research Laboratories, Santa Barbara, California.

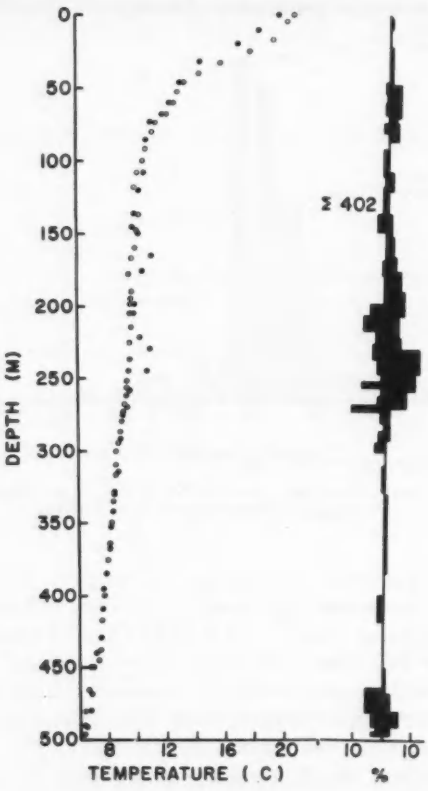

FIG. 10. Vertical distribution (percentages) of *E. pacifica* in the San Diego Trough, 32°42.7′N, 117°26.8′W, May 18, 1965, 1725 to 1815 local time, taken by Longhurst et al. (1966). Results for the descending plankton recorder are given on the left bar graph and the ascending on the right. The temperature profile shown at the far left was taken simultaneously.

short-term feeding experiments. It includes any carbon which may have been deposited in molts or reproductive products. This efficiency is analogous to the "gross growth efficiency" defined by Conover (1964) but with carbon substituted for weight or calories.

Assimilated carbon refers to the portion of ingested carbon which is digested by the euphausiid, and is analogous to Conover's "assimilation efficiency." The fraction of the assimilated carbon which appears in the tissues of the euphausiid due to growth is referred to as incorporation of assimilated carbon (Conover's "net growth efficiency") and can be distinguished from

the remainder of the assimilated carbon, which is lost in molts, reproductive products, or respired carbon dioxide.

Laboratory Carbon Budgets

From the percentage carbon determined for the dry weight of *E. pacifica* ($42.0 \pm 1.7\%$) the amount of organic carbon can be calculated for any size of individual. If increase in weight is known, therefore, the appropriate conversions yield information on the addition of organic carbon due to growth (carbon incorporation). Calculation of the carbon lost due to molting was based on actual molt weights obtained from laboratory animals and the amount of carbon that these molts contained. The loss of carbon due to molting in the field population was based on 10% weight loss every 5 days observed by Lasker (1964) and Jerde and Lasker (1966) and 17% organic carbon content of the molt. Respired carbon was calculated for *E. pacifica* during specific growth periods from the mean respiratory rate of 1.47 μliters O_2 per mg per hr and a respiratory quotient of 0.8 which assumes chiefly a fat and protein metabolism.

The carbon budgets for seven experimental animals (Table II) show that where growth is rapid incorporation of assimilated carbon is high and may account for as much as 30% of all the carbon entering the tissue of the animal. Where growth is slower, incorporation is as low as 6%. The long-term loss of carbon due to molting ranged from 6 to 11%; respiration accounted for 62–87% of the assimilated carbon.

Ponomareva (1963) stated that spawning of *E. pacifica* takes place in June in the Bering Sea and in the following winter two modal groups, presumably of successive year-classes, can be found at about 6.4 and 11.6 mm body length. In spring of the following year the two groups grow, respectively, to 10 and 15 mm body length. Using the weight–length relationship for fresh animals shown in Fig. 1, I converted Ponomareva's length figures to weight and calculated the carbon budget of the population (Table II), using laboratory data on molting and respiration. Because the respiratory need for carbon of the animal predominates over the life span, the incorporation of assimilated carbon is about 9%, not including eggs and molts.

Some information is available on reproduction of this euphausiid species. Ponomareva (1959) has counted the ova in *E. pacifica* from the northwest Pacific Ocean and has deduced that the fecundity is 1400 eggs. In a personal communication (1965) she has written me that spawning occurs only once in the life span of the species in the region of the Japan Sea, the Bering Sea, and the northwest Pacific Ocean. I obtained some *E. pacifica* eggs immediately after spawning. They weighed 5μg dry weight per egg, and contained about 50% C. A fecundity of 1400 eggs per female implies that 3.5 mg C were incorporated into ova during the life span of the female. The fraction of assimilated carbon calculated to appear in eggs was 9%. No information is yet available on the carbon needed by males for reproduction.

TABLE II. Weight and percentage incorporation of assimilated carbon by *E. pacifica*.

Animal No.	Initial dry weight (*mg*)	Final dry weight (*mg*)	Time (*days*)	Weight of assimilated carbon (*mg*)				Percentage of carbon incorporated			Percentage carbon used in respiration
				Growth	Molts	Respiration	Eggs	Growth	Molts	Eggs	
Laboratory animals											
1	0.30	1.19	69	0.37	0.10	0.78		30.0	8.0		62.0
2	0.30	1.20	73	0.39	0.07	0.83		29.6	5.7		64.7
3	0.50	1.50	69	0.42	0.11	1.04		26.8	6.8		66.4
4	1.56	2.30	51	0.31	0.15	1.49		15.9	7.7		76.4
5	0.80	1.36	66	0.24	0.094	1.08		16.7	6.6		76.7
	1.32	1.85	65	0.25	0.12	1.53		13.1	6.4		80.5
	1.95	2.31	23	0.15	0.066	0.84		14.3	6.2		79.5
6	1.53	2.00	63	0.20	0.24	1.68		9.4	11.3		79.3
7	1.65	1.94	63	0.12	0.14	1.71		6.2	7.0		86.8
Field population[a]											
	0.23	9.0	580	3.68	5.95	26.1	3.5	9.4	15.3	8.9	66.4

[a]Growth of field population based on data of Ponomareva (1963).

To summarize, over the 20 month span after hatching from the egg the average female *E. pacifica* in Ponomareva's study was calculated to respire 67% of its assimilated carbon and to invest 9% in growth, 15% in molting, and 9% in the production of eggs.

Experiments With Carbon-14

In earlier short-term experiments (24 hr) where *E. pacifica* were fed on unicellular algae, usually over 90% of the food was assimilated and the carbon incorporation efficiency ranged from 11 to 74% (average 32%) of the ingested carbon (Lasker, 1960). Because of their short-term nature these experiments included as "incorporated" any carbon which was mobilized into the molts of the animals and was destined to be descarded. The Q_{O_2} used then was 1.14 μliters per mg per hr, whereas a more reliable value obtained from many additional measurements, and the one used here is 1.47 μliters per mg per hr.

For *Artemia* nauplii to become sufficiently radioactive they must develop beyond the yolk stage and feed on suspended radioactive algae. Unfortunately, the *Artemia* have grown so large (to ca. 0.75 mm long) by the time they are sufficiently radioactive (5 days old) that *E. pacifica* has difficulty ingesting them. Consequently, almost all of the experiments (Table III) show assimilation of carbon from food which was not eaten in great enough quantity to meet all the metabolic needs of the animal. Four euphausiids (No. 8, 10, 13, and 14) ate enough or almost enough nauplii to satisfy their complete metabolic needs. Assimilation of ingested carbon by *E. pacifica* was high 84%; range 66–95%) in 23 out of 24 animals. One animal that assimilated only 46% is not included in the average because the food left in the foregut made up an abnormally large unassimilated fraction that could have been digested later.

A large fraction of the ingested carbon (average, 25.6%) appeared in the tissues of almost every animal in spite of the fact that there was usually insufficient carbon to satisfy all the carbon demands of the animal. Apparently, the euphausiid does not use all incoming carbon for respiration, but rather draws upon its own tissues to make up for any metabolic deficit. In those where sufficient carbon was supplied as food, short-term efficiencies were similar to those obtained by growth experiments in the laboratory where molt carbon was included as part of the incorporation of carbon.

FOOD AND FEEDING

A euphausiid must capture food particles of sufficient size and carbon content and at an adequate rate to satisfy its carbon requirements. Laboratory experiments have shown that *E. pacifica* is capable of eating from suspension either algae or small nauplii, and in her recent book Ponomareva (1963) listed a variety of phyto- and zooplankters found in the thoracic baskets of this species. In an earlier paper, Ponomareva (1955) stated that phytoplankton do not play an important role in the feeding of *E. pacifica* although some

TABLE III. Carbon budget of *E. pacifica* determined with carbon-14. Catabolic carbon = incorporated carbon + intestine carbon + egested carbon. All weights in milligrams.

Animal No.	Dry weight animal	Weight of carbon			Per cent C incorporated	Weight of carbon				Percent C assimilated
		offered as *Artemia*	ingested	incorporated		in intestine	egested	catabolized including CO_2	respired (calculated from RQ = 0.8)	
1	7.394	.0553	.0456	.0167	36.6	.0044	.0018	.0227	.101	86
2	7.668	.0553	.0308	.0036	11.7	.0016	.0001	.0255	.106	94
3	4.614	.0553	.0338	.0070	20.7	.0017	.0006	.0245	.067	93
4	3.515	.0553	.0406	.0142	35.0	.0021	.0008	.0235	.055	93
5	8.892	.1106	.0998	.0456	45.7	.0070	.0047	.0425	.146	88
6	7.360	.2765	.0915	.0200	21.9	.0081	.0133	.0501	.107	77
7	6.138	.2765	.1004	.0295	29.4	.0098	.0141	.0497	.089	78
8	5.747	.2765	.2317	.0919	39.7	.0119	.0244	.1035	.084	84
9	6.998	.2765	.0527	.0164	31.1	.0077	.0067	.0279	.101	73
10	6.028	.2765	.1496	.0255	17.1	.0105	.0020	.1116	.087	92
11	7.305	.2765	.0894	.0095	10.6	.0040	.0008	.0751	.106	95
12	8.072	.2765	.1403	.0246	17.5	.0143	.0034	.0980	.117	87
13	7.440	.2765	.1817	.0419	23.1	.0188	.0194	.1016	.108	79
14	9.522	.5530	.4206	.1686	40.1	.0529	.0238	.1753	.138	82
15	2.652	.0338	.0168	.0036	21.4	.0008	.0003	.0121	.040	93
16	4.978	.0553	.0317	.0098	30.9	.0050	.0019	.0150	.076	78
17	4.652	.0553	.0491	.0126	25.7	.0088	.0031	.0246	.037	76
18	6.554	.0205	.0180	.0083	46.1	.0046	.0016	.0035	.102	66
19	3.250	.0553	.0305	.0078	25.6	.0033	.0015	.0179	.047	84
20	2.555	.0553	.0245	.0076	31.1	.0024	.0011	.0133	.038	86
21	1.275	.0830	.0149	.0008	5.5	.0000	.0000	.0141	.020	80
22	2.033	.1106	.0497	.0076	15.3	.0255	.0013	.0153	.031	46
23	3.532	.0553	.0458	.0071	15.4	.0032	.0060	.0295	.056	80
24	4.601	.1106	.0557	.0053	9.5	.0039	.0073	.0391	.072	80
					Average: 25.6					Average: 84[a]

[a]Exclusive of animal No. 22; see text.

do feed on phytoplankton in the spring. She found that feeding is confined to the upper 100 m of water and occurs at night and in the evening. Deeper than 500 m there is little or no feeding.

Because *E. pacifica* is omnivorous, the feeding behavior of individuals on diets of unicellular algae and *Artemia* nauplii was examined. It became evident that several important factors affect feeding by *E. pacifica*.

Particle Size

The size of the food particle governs to a great extent whether a euphausiid will succeed in ingesting it. Individual *E. pacifica* were fed experimentally a known number of newly hatched *Artemia* daily except for 1 day when *Artemia* which were 5–6 days post-hatch (750 μ long) were presented to the animals (Table IV). *Euphausia pacifica* drastically reduced its intake when it was fed large nauplii. In 22 additional experiments where 500 newly-hatched nauplii were presented to individual animals the average number ingested was 52 nauplii per milligram dry weight euphausiid per day; in four experiments where 500 large *Artemia* nauplii were presented to individual euphausiids they ate on the average only five nauplii per milligram euphausiid per day.

Effect of Molting on Feeding

When *E. pacifica* molted during a feeding experiment fewer nauplii were eaten during the day of molt (Table IV). Thus, molting acts as a depressant to feeding.

Table IV. Feeding of individual *E. pacifica* on *Artemia* nauplii as affected by euphausiid molting and the size of the nauplii. All animals were fed newly hatched (0.375-mm) nauplii except on day 7 when 0.75-mm nauplii were substituted.

Animal No.	Dry weight of animal (*mg*)	Nauplii	Day 1	2	3	4–6	7	8	9	10
1.	3.519	No. offered	600	610	600	1010	500			
		Per cent eaten	33	54	5[a]	30	2			
2.	5.575	No. offered	500	500	500	1000	515	500	500	700
		Per cent eaten	34	39	7[a]	90	7	14[a]	30	18
3.	5.000	No. offered	1000	500	1015	1000				
		Per cent eaten	20	34	34	25[a]				
4.	1.836	No. offered		500	500	1020	500	500	500	700
		Per cent eaten		21	1[a]	49	1	7	10[a]	21
5.	4.578	No. offered		500	500	1000	500	500	500	700
		Per cent eaten		67	45	44	2	42	22[a]	23

[a]Molted.

Density of the Food Organisms

An adult *E. pacifica* can ingest all or nearly all of 50 *Artemia* nauplii in 100 ml of sea water over a 24-hr period in the dark. When the volume of sea water is increased while the number of nauplii is kept constant, the effect of density of food organisms can be determined and recorded as milliliters of water cleared per animal per hour (F). The animals chosen for this experiment were well fed and had molted 1 or 2 days earlier. The results (Fig. 11) indicated that the median "filtering rate" was highest (45 and 43 ml per animal per hour) at densities of one nauplius in 20 ml and 40 ml, respectively. However, a further decrease in density to one nauplius in 60 ml presented the animal with a more severe food-catching problem, and the median filtering rate diminished to 22 ml per animal per hour, although some euphausiids were still moderately successful. The high filtering rates obtained with diets of *Artemia* nauplii were never obtained in experiments where algae were fed. Euphausiids probably can sense and capture their prey and the so-called "filtering rate" is more properly a capture rate dependent upon factors other than random swimming and sweeping clear a volume of water as expressed in the Gauld equation. The decrease in density may impose a detection problem on the euphausiid by increasing the mean distance between it and its food particle. If so, then the critical distance between an adult *E. pacifica* and its food is about 1.7–2 cm, beyond which the euphausiid becomes inefficient at detecting and capturing prey in the dark.

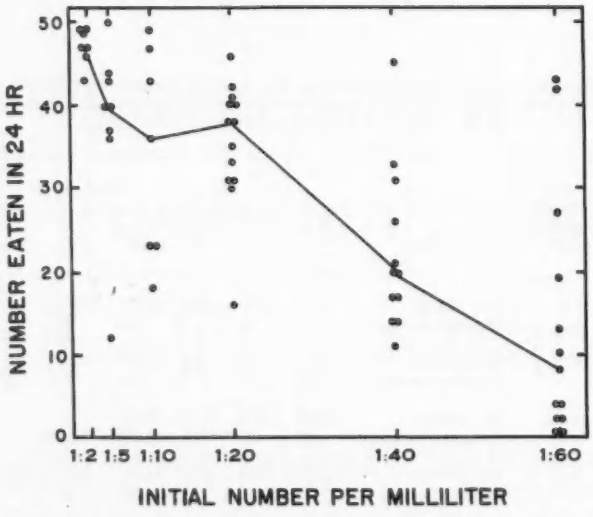

FIG. 11. The relation between density of *Artemia* nauplii and the number eaten by adult *E. pacifica*. Each animal was presented with 50 newly-hatched *Artemia* nauplii in sea water adjusted in volume to provide the densities indicated. The line is drawn through the median values.

Food Selection

Anraku and Omori (1963) found that some copepods, e.g. *Centropages hamatus* and *C. typicus*, although omnivorous, decreased their feeding on the diatom *Thalassiosira fluviatilis* to feed on *Artemia* nauplii available at the same time. *Euphausia pacifica* behaved similarly, showing distinct preference for nauplii over the diatom. In 17 experiments *Thalassiosira* was fed to *E. pacifica* at a concentration of 15×10^3 cells/ml; 500 *Artemia* nauplii were added in each of six of these; the filtering rates on *Thalassiosira* over 24 hr were recorded in both situations. Feeding on the diatom was depressed (median $F = 0.3$) over the controls (median $F = 1.9$) when *Artemia* was present ($P < 0.01$; Wilcoxin "*t*" test).

In another series of experiments, feeding on *Dunaliella tertiolecta* alone was alternated with feeding on *Dunaliella* plus 500 *Artemia* nauplii. The results (Table V) show that feeding on *Dunaliella* was suppressed or even eliminated when the euphausiid had brine shrimp nauplii available.

Euphausia pacifica may select among different copepod food organisms in the sea. Ponomareva (1955) compared the percentage composition of copepods in water being grazed by euphausiids with that in the thoracic baskets of euphausiids from the same water. Some copepod species, e.g. *Metridia pacifica* and *Calanus tonsus*, were eaten to a greater degree than their relative abundance in the water would give cause to expect. *Oithona similis*, a copepod of the proper size range for ingestion and present in large numbers, was rarely eaten.

Table V. Filtering rates[a] of *E. pacifica* on 15×10^3 cells/ml of *Dunaliella tertiolecta* in 500 ml with and without *Artemia* nauplii present. Days are consecutive.

Animal No.	Dry weight of animal (*mg*)	Filtering rate on *Dunaliella*		Number of nauplii eaten on day 2	Per cent eaten of all nauplii offered
		Day 1 *Dunaliella* alone	Day 2 *Dunaliella* and *Artemia*		
1	1.7	1.3	0.4	31	6
2	5.3	10.5	0.8	252	50
3	4.9	1.4	0.1	42	8
4	4.3	24.9	0	65	13
5	4.4	2.3	0	304	61
		Dunaliella alone			
6 (control)	3.8	2.3	1.6[b]	–	–

[a] Milliliters cleared per animal per hour.
[b] On the third day the filtering rate remained 1.6 for the control organism.

Individual Variability

An experiment was performed to observe the feeding characteristics of individual *E. pacifica* over relatively short time intervals. Four animals (A, B, C, and D, Fig. 12) were given 50 *Artemia* nauplii each in 100 ml of membrane-filtered sea water at 10 C (membrane pore size, 0.45 μ). Because molting depresses feeding (see above) only animals that had molted at least 24 hr earlier were used. These experiments were done only in the dark or under a red light when the nauplii were counted. Short-term variations occurred in the feeding rate and each individual appeared to feed differently. Animal A devoured nauplii at a steady rate, 1–2 nauplii/hr; animal B ate half of the nauplii in the first 4 hr and thereafter ate at a constant rate. Although not indicated by the ingestion curve in Fig. 12, animal C killed many nauplii without devouring them so that the curve should more closely approximate that of animal B; animal D ate more than half of the nauplii between the 8th and 12th hr of the experiment.

These results illustrate the variability of individual feeding in this organism. All subsequent experiments are presented as rates over a single day and therefore represent an average of differing short-term rates.

Particulate Feeding

I calculated how many nauplii of a size and carbon content equivalent to an *Artemia* nauplius (5.5×10^{-4} mg C/nauplius) must be captured and eaten

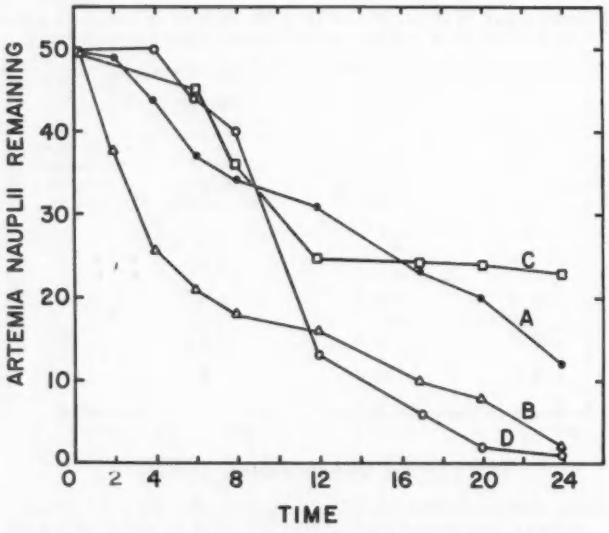

FIG. 12. Feeding of four individual adult *E. pacifica* on *Artemia* nauplii recorded at short intervals over a 24-hr period. The initial density of nauplii was 50/100 ml.

in the sea to satisfy the euphausiid's carbon requirements for growth, molting, respiration, and digestive inefficiency. The 0.24-mg juvenile needs 12 nauplii/day and the largest animal considered, 9 mg, must capture 336 per day (Table VI). Under laboratory conditions these numbers were easily captured because the density of nauplii was maintained as high as 4–5 nauplii/ml. Comparable densities have not been reported for the sea. The euphausiid may meet its food requirements by feeding on aggregations of food organisms as reported by Ponomareva (1954).

GRAZING

McAllister et al. (1960) investigated the primary productivity of the northeastern Pacific Ocean within the subarctic water mass, about 500 miles from the nearest part of the continental shelf, and in a region where *E. pacifica* is found in moderate abundance and near an area where maximum concentrations of this species have been observed (Brinton, 1962). During the summer period of productivity, they found detrital carbon equivalent to 0.1–0.2 mg C/liter and plant carbon between 0.01 and 0.035 mg C/liter. Production was about 0.007 mgC per liter per day. From the values of total carbon obtained by McAllister and coworkers I calculated (Table VI) the amount of grazing on phytoplankton and detritus required by individual *E. pacifica* of different weights to maintain their metabolism and growth in nature. If the total amount of particulate organic carbon available is considered as the food source, ca. 0.16 mg C/liter (detritus and phytoplankton) and uniformly distributed, a 0.24-mg animal would need to filter 43 ml/day (F = 2 ml per hr per animal)

TABLE VI. Nauplii required by *E. pacifica* and filtering rates on phytoplankton and detritus in ocean, to provide carbon needed at various levels of growth. Filtering rates based on 0.16 mg particulate carbon per liter in northeastern Pacific Ocean, summer (see text), calculated on basis of 90% assimilation of ingested carbon.

Dry weight of animal (*mg*)	C requirement/day in $g \times 10^{-5}$	*Artemia* nauplii needed per day	Milliliters of sea water animal must clear per hour to satisfy requirement
0.24	.68	12	2
1.0	2.4	48	6
2.0	4.6	96	12
3.0	6.6	120	17
4.0	9.2	168	24
5.0	11.0	192	29
6.0	14.0	264	36
7.0	15.0	264	39
8.0	18.0	336	47
9.0	19.0	336	49

and the 9-mg animal would need to filter 1170 ml/day (F = 49 ml per hr per animal). Filtering rates for intermediate animals are presented in Table VI.

Because *E. pacifica* is omnivorous it is likely that both detritus and phytoplankton are eaten when no prey, e.g. nauplii, are available. Experiments were performed, therefore, to indicate the ability of the euphausiid (dry weight = 2.8–5.1 mg) to filter water containing phytoplankton cells. The rates obtained (Table VII) varied considerably from animal to animal; the maximum rate was 38 ml/hr for a 4.3 mg animal on a dilute suspension of *Platymonas*. The average rate on *Platymonas* was 7 ml per mg euphausiid per

TABLE VII. Filtering rates of *E. pacifica* on unicellular algae. Twenty-four-hour experiments in 1 liter of sea water between 10 and 13 C.

Dry weight of animal (*mg*)	Number algal cells/ml	Filtering rate	
		Ml/hr	Ml/mg euphausiid/hr
Platymonas subcordiformis			
2.8	1,600	32	11.5
3.5	1,700	18	5.2
4.9	2,000	12	2.4
4.3	3,600	38	8.8
Gonyaulax polyedra			
5.1	6,400	17	3.4
3.8	6,500	20	5.4
3.9	14,700	6.7	1.7
Thalassiosira fluviatilis			
5.2	15,600	2.8	0.5
5.0	16,800	2.3	0.5
5.2	17,100	7.1	1.4

hr or 168 ml per mg euphausiid per day. On a diet of *Gonyaulax polyedra* the average rate for three animals was 3.5 ml per mg per hr or 84 ml per mg per day. Three animals feeding on *Thalassiosira* showed markedly lower filtering rates, averaging 0.79 ml per mg per hr or 19 ml per mg per day. The low rates with *Thalassiosira* seem to reflect rejection of this species by *E. pacifica*. Often no cells of this species of alga were eaten whereas cells of *Platymonas*, *Dunaliella*, or *Gonyaulax* were always taken.

In four experiments the number of *Thalassiosira* was adjusted (7.3×10^3 cells/ml) so that the total volume of the cells equalled the total volume of the cells of *Dunaliella* (41×10^3 cells/ml) used. (Cell volumes were determined by Mullin et al. (1966).) The filtering rates (milliliters cleared per hour) were:

Animal No.	Dry weight (*mg*)	Day and food 1 *Dunaliella*	2 *Thalassiosira*	3 *Dunaliella*	4 *Dunaliella*	5 *Dunaliella*
1.	3.0	2.2	0	1.7	1.5	–
2.	3.0	2.6	0	0.2	1.6	–
3.	2.7	–	2.0	3.7	1.6	0.6 (molted)
4.	3.8	–	0.4	2.3	1.6	1.6

Two of the euphausiids (No. 1 and 2), conditioned to feeding on *Dunaliella*, did not filter *Thalassiosira* the following day when no *Dunaliella* were present. *Dunaliella* was offered without *Thalassiosira* on the third day; one animal resumed feeding immediately but the other did not eat until the fourth day. Thus, a lag effect seemed to be induced by exposure to *Thalassiosira* in one animal. Euphausiids No. 3 and 4 commenced filtering when *Thalassiosira* was offered first. The filtering rate increased substantially the next day, when only *Dunaliella* was offered.

The filtering rates may be misleading as far as the capacity for food-getting by an individual euphausiid is concerned. In dense suspensions of algae the euphausiid can fill its foregut, gland, and intestine quickly so that effective filtration drops to practically nothing in a few minutes. On a diet of *Dunaliella* (10^6/ml) a euphausiid can pack its foregut and gland within 10 min. The first fecal pellet is formed and passed within 1 hr and if the animal is subsequently starved the foregut may still retain a trace of green plant matter after 2 days. If *Artemia* are caught and eaten by the euphausiid just after the digestive tract has been crammed with algae, digestion seems to be accelerated; within 6 hr no trace of chlorophyll remains in the digestive tract. Animals with full digestive tracts observed immediately after collection at sea did not fully empty their foreguts even after 24 hr without feeding. At minimal feeding (50 *Artemia* nauplii) some food was still in the foregut after 40 hr. When a surplus of nauplii was fed over the same period to other individuals of the same batch no trace of the former food remained in the foregut.

The total amount of particulate carbon available in the sea is adequate to maintain *E. pacifica* at the filtering rates determined in the laboratory. Phytoplankton cells alone would not suffice (Bainbridge, 1957). It is more likely, however, that *E. pacifica* depends in large part upon its ability to exploit aggregations of copepods than on its marginal ability to filter enough carbon from a dilute suspension.

GENERAL DISCUSSION

Several interesting features of euphausiid feeding were brought out in this investigation. Selective feeding is not new but now can be extended to the

euphausiids. These experiments give the impression that the euphausiid may have a great sensory capacity for detecting food organisms in the surrounding water. The thoracic basket of the euphausiid is its filtering mechanism. The effective opening into the basket of a 3.5-mg animal is 3 mm^2. An opening of this size can filter 21 ml/hr at a cruising speed of 2 cm/sec or 42 ml/hr at a cruising speed of 4 cm/sec. These cruising speeds were observed for this euphausiid in the laboratory. Occasionally, speeds up to 18 cm/sec were attained by adult animals but only for 1 or 2 sec. The aforementioned filtering capability permits achievement of the median filtration rates of 45 and 43 ml/hr, which were calculated for *E. pacifica* feeding on *Artemia* nauplii at a nauplii density of 1 in 20 ml and 1 in 40 ml, respectively. The median rate decreased sharply, however, when only one nauphius/60 ml was offered (Fig. 11); a sensory mechanism with a finite range may be used by the euphausiid for capturing nauplii.

Euphausia pacifica seems to become conditioned after feeding on a particular food and neglects other food organisms which are acceptable on other occasions, but less desirable. In several experiments a day or more elapsed before a euphausiid previously fed on *Artemia* began to feed on suspended algae. This behavior did not seem to be a case of "filling up" because feeding on *Artemia* continued unabated in densities of 2–3 nauplii/ml and digestion was rapid. Thus, the food available and the animal's inclination to feed on the food are factors which must be considered in relating phytoplankton carbon production to incorporation of this carbon into the zooplankton.

Two assessments of carbon transfer were made in this study. In the laboratory, rapidly growing *E. pacifica* can assimilate as much as 90% of the carbon they ingest and incorporate as much as 30% of the assimilated carbon into their tissue. This efficient utilization of carbon may never be realized for the majority of individuals in the ocean and the incorporation is probably nearer to 10% of the assimilated carbon.

Some observations were made on the molting of *E. pacifica* which are important to this discussion. Molting seems to be confined to the night time and, in laboratory containers, molts are degraded to a softened, easily torn condition within 24 hr, probably by bacterial action. The sinking rate of intact molts, determined in sea water at 10 C and 33‰ salinity, was about 1 m in 5 min. Thus, molts may drift down almost 300 m in a day when undisturbed. If euphausiid ecdysis in the sea occurs only at night, then molts which are released in the upper 100 m persist at least through 1 day between the surface and 400 m.

The carbon incorporated and released as part of spawned eggs represents no real loss to the planktonic food chain because the eggs remain available to grazers or develop further. If the molts are eaten, then the amount of organic carbon available to the next step(s) in the food chain is about 30% of that ingested throughout the life span of euphausiids. Should the molts drift out of the system uneaten, the carbon available to predators in the bodies of the

euphausiids and their eggs is about 19% of that which was orginally eaten by the euphausiids.

In an earlier paper (Lasker, 1964), I suggested that molts might contribute substantially to the detritus of the ocean. Detritus, as Krey (1961) stated, may contribute from 40 to greater than 90% of the organic matter in ocean regimes; McAllister et al. (1960) recorded that 80–90% of the organic matter in the northeastern Pacific Ocean was detrital. The chemical composition of detritus suggests that it is of animal origin (Parsons and Strickland, 1962).

The contribution to detritus by molting may be viewed in two ways. First, the 10% of the dry weight of each euphausiid shed every 5 days gives the figure of approximately one euphausiid biomass released to the sea every 50 days as molts. In 1 year this total may be seven times the euphausiid biomass. Secondly, Jerde and Lasker (1966), using the wet biomass figure for *E. pacifica* (1 g/m^2) supplied by Dr E. Brinton, calculated that 1.5 g per m^2 per year is dropped as molt dry weight over the euphausiid's range (1.3×10^{13} m^2). In terms of organic carbon this is 0.3 g C per m^2 per year or 0.4% of the actual carbon production of the euphotic zone in the summer as reported by McAllister et al. (1960). The facts that other euphausiid species contribute to the zooplankton biomass, that biomass estimates are low, that other crustaceans are molting, and that primary production is lower in the winter, indicate that 0.4% is a minimal figure.

Because of the regularity of molting it is tempting to suggest that molts might be used to estimate the biomass of euphausiid shrimps. The chief problem of biomass estimates from plankton net collections is the avoidance behavior of zooplankters (Fleminger and Clutter, 1965); molts do not present this problem. Molts are caught in plankton nets but usually are ignored later by the investigator. Yet this study has shown that two important features of a euphausiid besides its identification can be told from its molt: dry weight and body length. Only the tail of the molt need be caught with the uropods and telson intact. Thus, information on size, weight, and numbers of different euphausiid species may be ascertained in this way, given data on molt residence time in the water column.

The live biomass of *E. pacifica* alone over the area in which it occurs in the Pacific Ocean is equivalent to 0.11 g C/m^2. About 5% of the carbon in the standing crop of *E. pacifica* is needed to maintain the growth, respiration, and molting of the population per day; therefore, 5.5 mg C per m^2 per day is ingested by the population. The mean daily primary production of the euphotic zone in the summer in the northeastern Pacific Ocean was reported by McAllister et al. (1960) to be about 205 mg C per m^2 per day. Therefore, about 3% of the production is ingested by *E. pacifica* alone, but not until the carbon has passed a previous step in the food chain, i. e. through a smaller, herbivorous crustacean.

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Premating and Mating Behavior of the Dungeness Crab (*Cancer magister* Dana)[1]

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ABSTRACT

A male and female Dungeness crab (*Cancer magister* Dana) were kept under continuous observation in an aquarium to observe the premating embrace, female ecdysis, and mating. The male held the female in premating embrace from June 10 until June 18, 1965, when she molted. The male allowed the female to reverse her position from the premating embrace, sternum to sternum, to that of female carapace to male sternum only after she became aggressive. The male never allowed the female to escape from his control while she molted. Copulation occurred 1 hr and 32 min after female ecdysis, when the new exoskeleton had firmed somewhat, and the male carried the female in a postmating embrace for 2 days.

Extensive wear on the chelipeds of male crabs may reflect prolonged periods in the premating embrace and stroking of the female by the male rather than matings with several females. The male crab probably becomes aware of the female's readiness to molt by her aggressive pinching of maxillae and eyestalks.

INTRODUCTION

Little has been written about the premating and mating behavior of the Dungeness crab (*Cancer magister* Dana). Since female molting occurs before mating, considerable speculation has occurred among biologists on how this is accomplished and on whether the male crab releases the female from the premating embrace when she molts. MacKay (1942) stated that mating seemed to be between a soft-shelled female and a hard-shelled male and believed that during molting the male released the female and reclasped her later. Cleaver (1949) stated that mating generally followed quite closely the procedure described by MacKay. Butler (1960) described copulation but was uncertain whether the male continued to hold the female throughout ecdysis.

The objective of the present study was to follow the molting and mating sequence of Dungeness crabs to completion.

MATERIALS AND METHODS

On April 21, 1965, five female crabs were placed in an aquarium with a moderate-sized male. Five days later the latter was replaced by a 178-mm male.[2] On June 7, three additional females, reportedly being clasped by males in a premating embrace when captured, were added to the aquarium. On

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[2]Straight-line measurement between the bases of opposite 10th anterolateral spines.

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the morning of June 10, the male crab was found clasping a 110-mm female in the premating embrace. These two animals were then transferred to a 50-gal aquarium supplied with a continuous flow of filtered sea water. The aquarium was partially covered to reduce the light intensity yet still allow observation of activities within the tank. Water temperature in the aquarium during the period of observation ranged from 12 to 15 C — slightly higher than the ocean during the same period. A 16-mm Bolex movie camera and 35-mm still camera were mounted on tripods and readied for photographing the molting and mating. Observations of behavior were made for a continuous 192 hr. All actions and behavior mannerisms were recorded.

RESULTS

During the period April 21 to June 7, 1965, the male crab in the aquarium ignored the females. On June 7 when the new females were introduced the male became quite active. He approached the new females, body held high, walking on the extreme tips of the walking legs. At this time and for the next 2 days the females resisted his advances. On the morning of June 10 the male was observed holding a female in the premating embrace (Fig. 1). At the time of transfer to the larger aquarium the male released the female, but within 3 min reclasped her. This was accomplished by walking toward her on the tips of his walking legs and grasping her with both chelipeds, then turning her sternum towards him and pulling her firmly to him. Throughout this activity the antennae of each animal vibrated. After clasping the female, the male walked about with her in his grasp seemingly to investigate the new quarters.

The female was observed on many occasions to make movements as if to free herself from the premating embrace. Each such movement resulted in her being pulled roughly back into position with an audible click of sternum upon sternum. Following each of these attempts to free herself, or whenever she became restless, the male would stroke her carapace with his chelipeds in an up and down motion that seemed to pacify her. This reaction was noted periodically during the entire time that she was in the premating embrace. The female repeatedly moved her chelipeds above and around the eyestalks of the male, but these movements did not appear to be aggressive in nature. On occasion the male would rise up on the tips of his walking legs and raise the female up into an elevated position nearly 6 inches off the bottom of the tank. This movement would be accompanied by a continuous flexing of the male's abdominal flap. The female on numerous occasions was noted rocking back and forth in the clasp of the male and on one occasion she flexed all legs simultaneously and nearly escaped from the male's clasp. His reaction to this was to move her roughly back into the premating position. On one occasion the male raised up on the tips of the walking legs and allowed the female to drop down on the bottom of the tank. He then pressed forward with abdominal flap flexing. After about 3 min the female started moving and the male

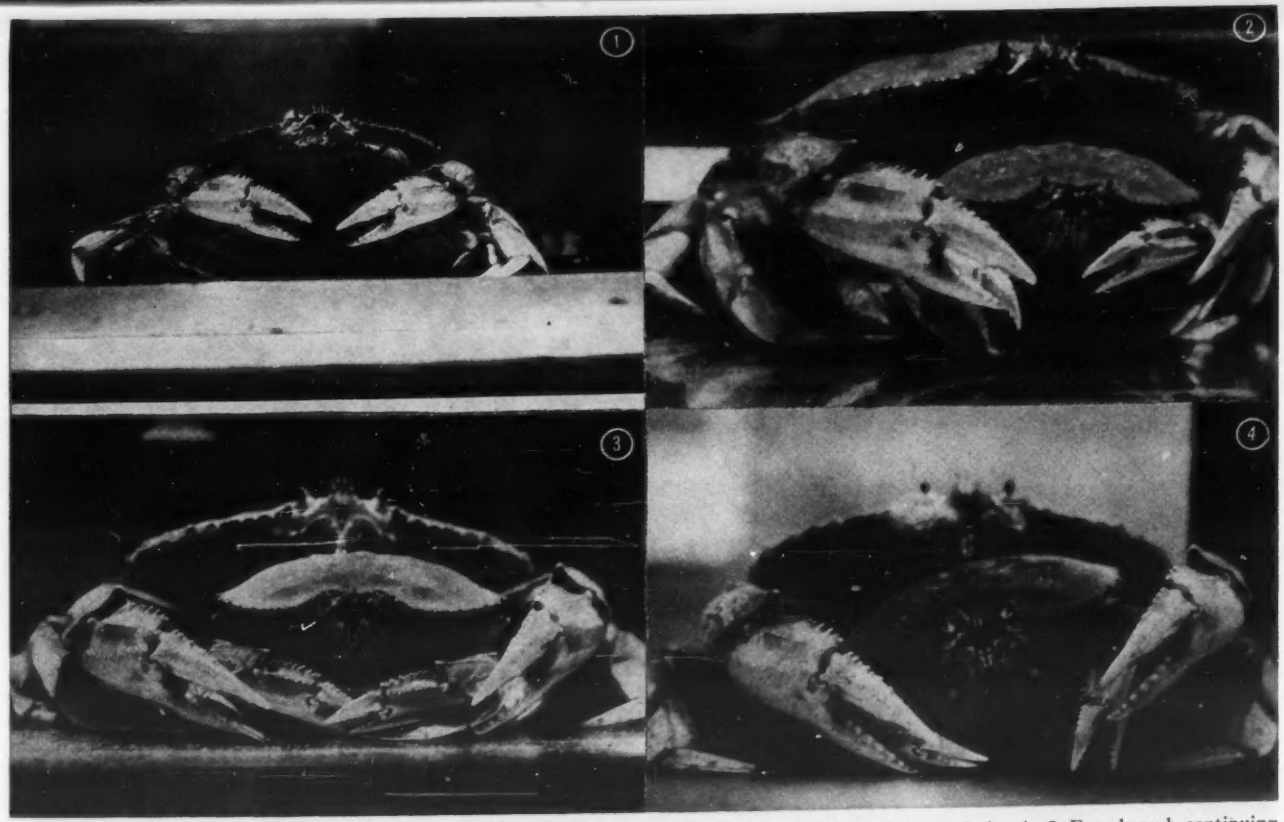

FIG. 1–4. **1.** Female crab in the premating embrace of the male. **2.** Female crab starting ecdysis while in control of male. **3.** Female crab continuing ecdysis while encircled by male's chela and walking legs. **4.** Female raising up and straining to start withdrawal from old exoskeleton.

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FIG. 5–8. **5.** Side view of female withdrawing from old exoskeleton. New carapace now exposed to 7th and 8th anterolateral spines. **6.** After female's withdrawal from the old exoskeleton, the male has shoved the old exoskeleton away. Female can be seen beneath male. Note the position of male mouth parts as compared with Fig. 3–5. **7.** Frontal view of copulating Dungeness crabs. **8.** Posterior view of copulating crabs showing position of the abdominal flaps.

reclasped her in the premating embrace. The male frequently walked around with the last pair of walking legs extended straight back.

Periodically we removed the aquarium covering to take photographs. At such times the male appeared to become agitated and would start walking about as if looking for an escape route. On one occasion, when the aquarium was covered, both animals rested in one corner of the tank with their eyestalks retracted and appeared to be sleeping. This behavior lasted for about 1 hr.

During the 5th day of observation (June 15, 1965) the male allowed the female to slip back to the vicinity of the 3rd and 4th walking legs. During one such period of positioning, the female attempted to escape and ended up with her carapace against his sternum. The male managed to turn the female again into the premating position after their positioning had been reversed for about 30 min.

During the 7th day of observation the female changed from relatively passive to active behavior. She attempted to push away from the male with the 3rd and 4th walking legs and nervously worked her walking legs and chelipeds. By 5:15 PM on June 17 the female's epimeral line obviously opened and both animals exhibited a new behavior pattern in that the antennae and mouth parts began moving rapidly. The male at this time allowed the female to hold herself 3–4 inches from his sternum but continued to hold her in his clasp. The female's behavior at this time became more aggressive and she started to grasp at the male's eyestalks and mouth parts with both chelae. This caused the male to pull her roughly back to his sternum. By 7:18 PM the female's suture line had opened up past the 10th anterolateral spine. At 7:50 PM the male lowered his grip to the base of the female's carapace and raised up as if to lift off the female's carapace. During this time the female continued her aggressive grasping at the male's maxillae and eyestalks. At 8:45 PM she managed to turn over in the male's clasp and ended up with her carapace to his sternum. At 9:21 PM the male raised up on the tips of the walking legs, grasped the female with his chelae, and again turned her over into the premating embrace. The female again started the aggressive movements and the male vigorously stroked her carapace. This aggressive activity by the female continued for about 2 hr; she then settled down into a passive state for about 2 hr.

At 2:00 AM on the 8th day she began actively trying to escape the male's clasp and grasping at his eyestalks and mouth parts. By this time her epimeral line had opened to the 2nd and 3rd anterolateral spine. At 2:17 AM the male allowed her to turn over so that her carapace was next to his sternum. Shortly after this reversal of position the male adopted a new position forming a "cage" with his walking legs and chelipeds that enclosed the female and kept her confined beneath him. By 3:29 AM the female was observed to be straining and moving her legs and appeared to be starting to withdraw from her old exoskeleton. During this period the male exercised only a moderate restraint over the female and even raised up to where she could move about underneath him. By 4:00 AM the female was definitely withdrawing from the old exoskeleton while completely encircled by the male's chelipeds and walking

legs. The sequence of the female molting is shown in Fig. 2–5. At this time it was again noted that the male's mouth parts and antennae were moving vigorously and whenever the female moved as if to get away, he would gently but firmly return her to the encirclement of his legs. By 5:45 AM the female's new carapace was bulging out to the 8th anterolateral spine and at 5:55 AM the female had nearly freed herself from the old exoskeleton. At this time the male raised up on his walking legs seemingly to facilitate the female's withdrawal. At 5:58 AM the female freed herself from the old exoskeleton and the male shoved it away with his chelipeds (Fig. 6). Following her withdrawal from the old exoskeleton the male hovered over the back of the female but did not attempt to pick her up. Eight minutes after the female finished molting, the male picked the female up in his walking legs, rolled her about and then released her. This action on the part of the male was repeated periodically for 1 hr and 22 min following her molting. At the end of this time he attempted to copulate, however, she slipped sideways from his grasp and he again hovered over her. Ten minutes later the male again turned the female over and she extended her abdominal flap and the male settled into the copulatory position with the gonopods inserted in the spermathecae (Fig. 7–8).

Copulation was the same as described by Butler (1960). However, after 12 min the male released the female when we tried to move the animals into a better photographic position. They were then placed in a darkened aquarium and after 30 min the male again mounted the female and remained in the copulatory position for 2 hr. Following copulation the male carried the female in a postmating embrace for 2 days before releasing her. This, as far as we are aware, is the first time that a postmating embrace has been recorded in Dungeness crab mating.

DISCUSSION

Though the conditions under which these observations were made were not natural and constitute a single observation, certain conjectures can be made. It appears highly improbable that the male crab ever lets the female out of his control once he has picked her up in the premating embrace. This might be particularly true in an environment where competing males could take her from him. At no time during these observations was the female capable of escaping the male's restrictions on her movements, even during ecdysis. During this latter period he allowed her to turn around but kept her in control and appeared to raise up in order to allow her to withdraw from the old exoskeleton. Following the female's molting the male did not attempt to copulate for 1 hr and 32 min; it appeared that he was waiting for a firming of the new carapace. Cleaver (1949) and Butler (1960) report that copulation takes about 30 min. Possibly the extended period of copulation observed here (2 hr) and the 2-day postmating embrace, which had not been reported before, are attributable to the unnatural surroundings and disturbance after the first copulatory attempt.

Butler (1960) concluded that extreme wear on the chelipeds of mating male crabs indicated prolonged periods in the premating embrace with several females. The male crab observed in this study did not have mating marks prior to picking up the female. At the end of 8 days he had pronounced marks on the wrist or carpus. These were undoubtedly created by the frequent stroking of the female carapace. The location of wear on carpus or propodus is probably dependent upon the size of the two animals involved. It appears that polygamy might cause heavy wear but it may also occur during a single mating if the male carries the female for an extended period of time.

Shellfish biologists have wondered how the male crab sensed that the time was right to release the female from the premating embrace so that she could molt. During our early observations the female remained relatively passive in the male's clasp, but during later stages she became rather aggressive and would pinch the male's maxillae and attempt to pinch the eyestalks. This behavior may cause a reaction in the male which allows the female to reverse her position in the premating embrace and complete ecdysis.

ACKNOWLEDGMENTS

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Incidence of *Gaffkya homari* in Natural Lobster (*Homarus americanus*) Populations of the Atlantic Region of Canada[1]

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ABSTRACT

Hemolymph samples drawn from 2035 lobsters captured in five areas off the Canadian Atlantic coast were individually tested for the bacterium *Gaffkya homari*, using a qualitative, presumptive test, followed by a confirmatory procedure. Ninety-six lobsters, or almost 5% of the total, were confirmed as being infected. The approximate range of *G. homari* incidence was from 2 to 40% indicating that *G. homari* is resident in the natural lobster population of this region. Evidence suggested that a high natural incidence can lead to an epizootic among stored, captive lobsters. The possibility that an infection source exists in addition to the *G. homari* strains resident in the lobster population is discussed.

INTRODUCTION

Snieszko and Taylor (1947) described a bacterial disease which caused heavy mortalities among captive lobsters (*Homarus americanus*) in Maine. These authors isolated the causative organism and Hitchner and Snieszko (1947) described it in greater detail and suggested the name *Gaffkya homari*. The capacity of *G. homari* to infect the European lobster (*Homarus vulgaris*) is evident from the epizootics recorded in Norway and the Netherlands (Roskam, 1957), Ireland (Gibson, 1961), and the United Kingdom (Wood, 1962, 1965). Roskam (1957) coined the term "gaffkaemia," referring to the apparently septicemic nature of the infection.

A preliminary report by Stewart and MacDonald (1962) suggested that *G. homari* was carried by the American lobster in its natural environment. Evidence tending to confirm this has recently been reported by Rabin (1965) for lobsters in the Woods Hole, Massachusetts, coastal region.

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The previous reports with the exception of those by Stewart and MacDonald (1962) and Rabin (1965) have not indicated the source of infections but rather have recorded only the last stages of the disease among animals held captive. The intention of the present study, prompted by outbreaks of gaffkaemia in the Canadian Atlantic region, was to confirm and extend the preliminary work of Stewart and MacDonald in determining whether the lobster population in its natural environment carried *G. homari*. In addition, information on the incidence levels and the extent to which these levels were related to gaffkaemia epizootics among captive lobsters was sought. A qualitative, presumptive method for the relatively rapid screening for *G. homari* at any stage of the infection was assessed.

MATERIALS AND METHODS

Lobsters

Lobsters, brought directly ashore from the fishermen's traps and judged by the commercial operators or the authors to be suitable for storage and subsequent sale alive, were used as the source material. The location of the five areas from which samples were obtained are shown in Fig. 1. With the exception of 100 lobsters in area 4 which were taken from traps set for 72 hr, no lobsters came from traps which had been set for more than 48 hr and in most instances the traps had been set for approximately 24 hr. Each lobster was examined and a hemolymph sample drawn within 1 hr of the time the

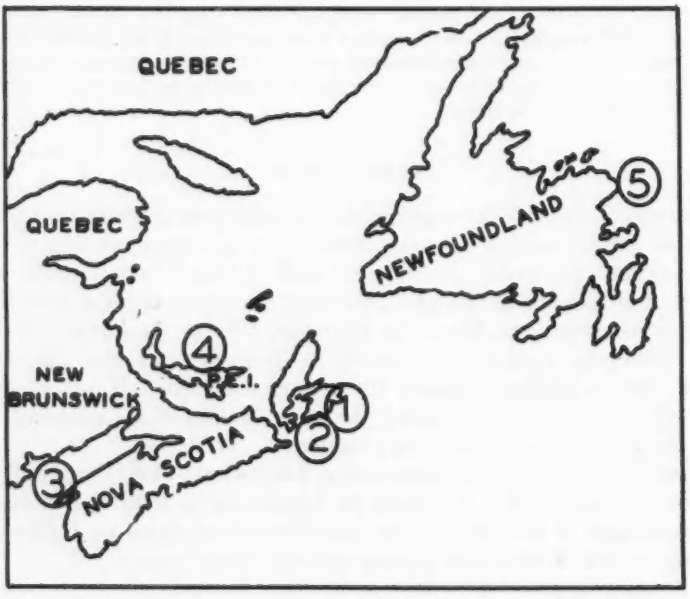

FIG. 1. Locations of the five areas sampled to determine the incidence of *G. homari* among lobster populations.

animal had been brought ashore. Usually the lobsters were returned immediately to the commercial operators to be included in their regular shipments. However, in area 4, after the samples had been drawn the lobsters were purchased and held captive to determine whether stress associated with commercial storage conditions contributed to outbreaks of the disease. A total of 1228 of the original 1470 lobsters arrived at the holding unit in good condition, the remainder because of weakness, mutilation, or death were not included in the holding experiment. These lobsters (average weight 245 g) with 28 of the infected animals randomly dispersed throughout the group were then held for approximately 4 months under a range of conditions common to the commercial storage of live lobsters.

Presumptive Test

The medium used in the presumptive test was composed of glucose, 6.5 g; Bacto yeast extract,[3] 4.5 g; tryptone, 15.0 g; NaCl, 6.4 g; phenyl ethyl alcohol, 2.5 g; brom-cresol purple, 0.008 g; dissolved in distilled water, 1000 ml; adjusted to *p*H 7.4, and dispensed in 4.5-ml amounts in screw-capped tubes and steam-sterilized for 15 min at 15 psi. In preliminary tests this medium was found to be suitable for the growth of *G. homari*.

A hemolymph sample, 1.5 ml, was drawn aseptically from the ventral abdominal sinus of each lobster and 0.5 ml was dispensed immediately into each of two tubes of the phenyl ethyl alcohol medium. These tubes were immediately shaken vigorously and then kept between 20 and 25 C (for several hours) until they could be incubated at 28 C. The hemolymph remaining in the syringe was used to make duplicate smears which were air-dried, heat-fixed, and stored for later examination as Gram stained preparations. The culture tubes were examined twice daily and those showing a change in color from blue to yellow, indicating acid production, were declared positive in the presumptive test.

Confirmatory Tests

The positive tubes from the presumptive test were used to streak BBL phenyl ethyl alcohol agar[4] plates which were incubated at 28 C, the incubation temperature of all the confirmatory work. Pinpoint colonies appearing on the plates after incubation were picked into Bacto brain heart infusion broth. The broth cultures were used to streak defibrinated sheep red blood agar plates and brain heart infusion agar slants. Blood agar plates not showing beta-hemolysis by the 5th day of incubation were declared negative. The growth on the agar slants was used for the catalase test and for a final Gram stain. The detection of catalase negative, beta-hemolytic, pinpoint colonies of Gram positive cocci occurring singly, paired, or in tetrads or other small groups, but

[3]Difco Laboratories Inc., Detroit, Michigan.
[4]Baltimore Biological Laboratory, Baltimore, Maryland.

not in chains or grape-like clusters, was taken as positive evidence of infection of the lobster by *G. homari*.

RESULTS AND DISCUSSION

The problem inherent in determining *G. homari* infections by an examination of a hemolymph smear alone is demonstrated in Fig. 2. In the initial stages (Fig. 2A) it is impossible to determine whether a lobster is infected. Prior to the midpoint of the infection, a few bacteria are occasionally noted but the hemocytes are still numerous (Fig. 2B). Between the mid infection and terminal stages of the infection the bacteria become predominant (Fig. 2C) and when the terminal stage or death is reached hemocytes are rarely seen (Fig. 2D). The tetrads and pseudocapsules typical of a heavy *G. homari* infection can be seen clearly in Fig. 2C and D. The time from the lobster's initial infection with *G. homari* to death can range up to 25 days or even longer depending as it does on environmental temperatures, size of the inoculum, and other factors. The effects of these factors will be reported in detail in a subsequent paper. A consequence of this is that a considerable period exists in which an infected animal, by an examination of its hemolymph smear alone, could easily be declared negative. The method of capture and the subsequent selection of lobsters for live storage tends to exclude animals weakened by the advanced stages of gaffkaemia. Thus, it is not surprising that direct microscopic examination of the Gram-stained hemolymph smears made in parallel with the presumptive test failed to detect *G. homari* in any of the lobsters.

In contrast, the cultural procedures used did demonstrate the presence of *G. homari* in the hemolymph of some of the same lobsters (Table I).

Accurate information concerning the outbreaks of gaffkaemia in commercial operations is difficult to obtain. Therefore, it is not always possible to link high natural incidences of infected lobsters with these outbreaks. However, the manager of a commercial holding unit acquiring live lobsters for storage at ambient temperatures from regions of known high natural incidences (areas 1 and 2; see Fig. 1 and Table I) reported mortalities ranging up to 50% of the captive stock only 2 weeks after the sampling date. Hemolymph taken from dead and dying lobsters in this unit was identical with that shown in Fig. 2D.

However, the mere presence of *G. homari* among captive lobsters does not necessarily mean that an outbreak of gaffkaemia is imminent. Evidence for this was obtained from the experiment in which lobsters from area 4 (see Fig. 1 and Table I) were held captive. Fifty-five per cent of the animals died during the 4-month storage period. The majority of the deaths resulted from weaker and molting lobsters being cannibalized. Cannibalism was such a serious factor that hemolymph smears were obtained from only 26% of the dead lobsters. No evidence of *G. homari* was found in any of these smears. Although not all of the dead lobsters could be examined because of cannibalism the fraction that was examined is large enough to be representative and permit the conclusion that *G. homari* was not a factor in the deaths. In addition 12 of the 28 infected

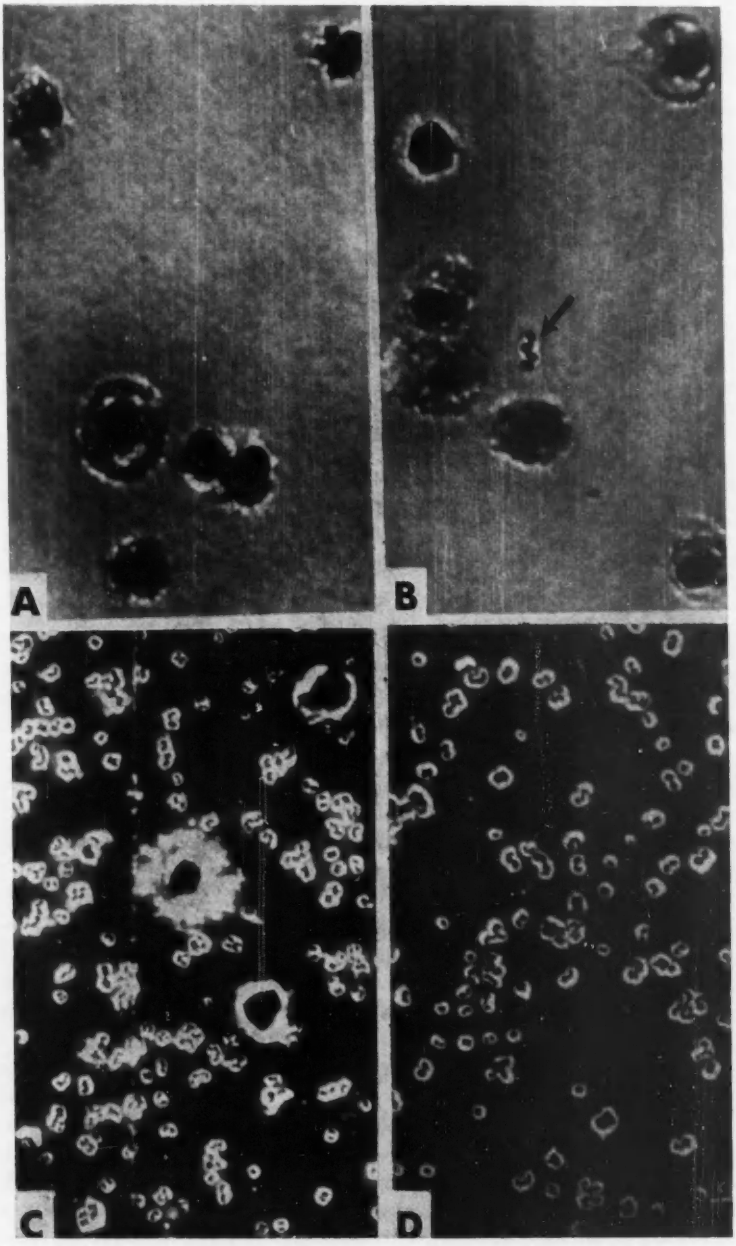

FIG. 2. Typical Gram stained hemolymph smears obtained at different times in the incubation period from lobsters infected with *G. homari*. A. Initial stage showing typical hemocytes alone. B. Early to mid-infection stages. Arrow indicates the few bacteria present. C. Between mid-infection and terminal stages. D. Terminal stage. × 530.

TABLE I. Incidence of *G. homari* among lobsters from the five sampling areas as determined by the cultural method.[a]

Area	Sampling date	Number sampled	Presumptive positive	Confirmed positive	Confirmed as per cent of total sample
1	July 1962	103	62	41	39.9
2	July 1962	83	60	8	9.7
3	July 1962	99	40	15	15.1
4	June 1964	1470	277	39	2.7
5	July 1965	180	54	3	1.7
Total:		2035	493	96	4.7

[a]Direct microscopic examination of Gram stained hemolymph smears made from the same hemolymph samples used in the cultural method failed to detect *G. homari* in any of the lobsters. Lobster ground temperatures (60 ft depth of water: 2–4 temperature readings per month for 5-yr periods) ranged from approximately 6 to 12 C for areas 1 and 2 in July and from 4 to 11 C in area 4 in June. Water temperatures for areas 3 and 5 are not available but would be expected to be similar.

animals survived and showed no evidence of *G. homari* in their hemolymph when they were retested by the cultural method at the end of the 4-month holding period.

It is highly probable that a variation in the virulence of *G. homari* resident in the population or a change in the resistance of the lobsters due to changes in the environment or both could result in high mortalities as Snieszko (1964, 1965) has suggested for the epizootics of fish diseases. Rabin (1965) speculated that the virulence of *G. homari* strains might be "a development of the conditions of the holding impoundment." The isolation of *G. homari* from shrimp in the Gulf of Mexico (Liuzzo et al., 1965) and the ability of *G. homari* to function well in NaCl concentrations ranging up to 7% (Aaronson, 1956) and its tendency toward halophilism as the incubation temperature is increased (Goldman et al., 1963) indicate that *G. homari* is well suited for a marine environment. Therefore, the original infections could stem from *G. homari* strains varying widely in virulence and resident in sources other than lobsters. Consequently, further studies on the epidemiology of this disease should not be restricted to lobsters.

Table I indicates that many lobsters carry other bacteria in addition to *G. homari* in their hemolymph. While the inclusion of phenyl ethyl alcohol in the presumptive test medium makes it selective for Gram-positive bacteria, it does not exclude entirely the Gram-negative bacteria, particularly if the incubation period is prolonged. Any bacteria capable of growing in the presence of phenyl ethyl alcohol and producing acid from glucose could give false positive results in the presumptive test (Cornick and Stewart, 1966). The failure of

G. homari to form tetrads consistently in laboratory media and the presence of other bacteria makes the confirmatory tests necessary. The presumptive procedure does permit the rapid sampling of a large number of lobsters with a reasonable degree of selectivity and the entire procedure was assumed to give a relatively accurate determination of the incidence of *G. homari* in the lobster population.

An examination of the areas located in Fig. 1 and the data in Table I shows clearly that *G. homari* is widespread in the Canadian Atlantic region. The incidence levels vary from one area to another and would be expected to vary with time within the individual areas. The fact that *G. homari* was found in all the areas tested would indicate that the bacterium is resident in the lobster population, although presumably it is usually at a low level.

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Perception of Surface Waves by the Blackstripe Topminnow, *Fundulus notatus*[1]

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ABSTRACT

The function of the cephalic lateral line in perceiving surface waves and its ecological significance was examined in the topminnow, *Fundulus notatus* (Rafinesque), a fish which frequents the surface waters. The sense organs are located in groups on the dorsal surface of the flatish head and are prominently visible on the skin. The individual organs of each group form a specific angle when related to the fish's midline. These organs perceive the force of slight surface waves and the perceptional field is omnidirectional. A great number of enucleated fish respond spontaneously, but when trained they orient toward the source of the disturbance on the surface up to a distance of 15 cm. The fact that the radiating wave is curved enables fish to locate the origin accurately. *Fundulus notatus* does not distinguish between two waves of equal strength striking simultaneously; it does so, however, if a time or intensity difference is present. A fish with sense organs removed from one side of the head deviates at a constant angle from the wave source. The perceptional field of individual organs or canals circumscribes a line drawn through the longitudinal axis of the organ or canal. A wave, therefore, traveling parallel to the longitudinal axis of the oval sense organ and hence the cupula, stimulates to the greatest extent whereas a wave perpendicular to it does not. The individual organs possess a directional property and, therefore, their arrangement on the head is functionally meaningful. The lateral line supplements the vision of the fish in finding its prey at the water surface.

INTRODUCTION

Because of the interesting variety in structure, function, and development of the lateral line system and its favorable external position, a large number of morphological, histological, and physiological studies have been made. Several papers are of historical importance (Parker, 1904; Hofer, 1908; von Frisch and Stetter, 1932; Hoagland, 1933; Dijkgraaf, 1934). Recent reviews critically evaluate the knowledge about it (Dijkgraaf, 1952, 1963; Lowenstein, 1957).

Since the lateral line and the inner ear are derived embryologically from a common anlage and since morphologically the two sense organs are very similar (often considered as the acoustico-lateralis system), there is little understanding of their physiological and functional relationship. In fact, controversy still exists as to what constitutes an adequate stimulus for the organs of the lateral line.

Well documented evidence (Hofer, 1908; Kramer, 1933; Dijkgraaf, 1934, 1947; Sand, 1937) shows that the sense organs of the lateral line are

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sensitive to water currents. The capability to detect moving objects (prey, predator) by means of the local water current is called "distant touch" or *Ferntastsinn* (Dijkgraaf, 1934). On the other hand, Parker (1904), Suckling and Suckling (1950), Jielof et al. (1952), Kuiper (1956) have demonstrated that the lateral line is sensitive to low-frequency vibrations (up to 130–200 cps). In continuing these studies, Harris and von Bergeijk (1962) confined the "hearing ability" of the lateral line in *Fundulus heteroclitus* L. to the "near-field of an underwater sound source." The authors achieved positive responses to low frequency (50–333 cps) sound waves if the distance from the sound source to the fish was small compared to the wavelength. Görner (1961, 1963), in an electrophysiological study, discovered that an individual sense organ of the lateral line of the clawed toad, *Xenopus laevis* Daudin, has a strong directional characteristic and, more recently, Schwartz (1965) reported results of a behavioral study in which it was shown that the same situation exists in the topminnow *Aplocheilus lineatus* Cuv. and Val. According to these authors, the shearing force arising from the bending of the sensory hairs is most probably the physical stimulus for the sensory cells.

In previous studies, benthic and pelagic fish were used for a specific objective, not necessarily emphasizing ecological factors. We chose *Fundulus notatus* Raf. (Cyprinodontidae) because of its habit of living close to the surface film. An obvious question is: What kind of adaptation do these fish have which enables them to pursue a livelihood at the surface? Our initial study dealt with the adaptation of the lateral line system and the question was asked: Does this surface-seeking fish perceive surface waves? An extensive study of the literature revealed that this question had not been examined, although Kramer (1933) and Dijkgraaf (1947) mention briefly that *Xenoplus laevis* reacts mildly to disturbances of the water surface.

MATERIAL AND METHODS

Material

The experimental animal, the blackstripe topminnow, *Fundulus notatus*, is native to southeastern Wisconsin (Greene, 1935). The experimental stock was obtained from the natural habitat and kept at room temperature (22 C) in an aquarium. This species occurs in relatively clear waters along the shores of lakes, ponds, and streams, and it prefers the quiet waters.

The blackstripe topminnow is a small, slender fish with a somewhat elongated body, compressed posteriorly. The top of the head is conspicuously flat, the dorsal fin is inserted caudal. Thus, the back appears to be straight. The mouth "opens along the upper surface of the head" (Hubbs and Lagler, 1957). We conclude that these structures are morphological adaptations for its distinctive surface-feeding behavior.

Methods

In a study of the function of the lateral line, precautions must be taken to eliminate cues to the eyes. Fishes were, therefore, enucleated under anaes-

thesia with tricaine methanesulfonate (M.S. 222—Sandoz). Only an occasional fatality occurred. Most operated fish began to feed on the day following enucleation. The enucleated animals were held individually in small aquaria and disturbances were avoided. For training and testing, a fish was transferred, in water, to a large experimental tank. The fish adapted readily to this handling and the experiments began promptly.

The experimental tank, lined with plastic, was square and had gently sloping walls. These flat walls were roughened and covered with a layer of fine sand in order to reduce reverberations of surface waves.

Surface waves were produced by touching the water surface lightly with the tip of a glass rod. A special holder provided a constant strength of the stimulus in some experiments. A few fish did not respond spontaneously, but, through training, soon learned to associate a food reward with small surface waves. The responses became more precise and dependable after a few days of training and henceforth it was only necessary to reward a fish with food following each fourth or fifth trial without a decrease in accuracy. Normally we carried out 190–260 trials in order to calculate the percentage response.

Two simultaneous surface waves of equal strength were created by a bipronged fork, whose tips (2.5 mm diameter) were 100 or 150 mm apart. In order to avoid movements other than vertical, the fork handle was placed firmly in an iron tube 150 mm in length. The tube was attached to a stand. A spring between the upper end of the handle and the tube kept the fork poised immediately above the water. After the fork was dipped into the water, barely breaking the surface, the spring's tension returned the fork quickly to its normal position. A careful adjustment of the fork assured simultaneous waves. Waves of different intensity were caused by using tips of greater thickness. Exactly beneath the fork and on the bottom of the tank, the two shadows of the tips were sketched as were their connecting and median line. Thus, a fish could easily be located at any time within this field.

The best method for removing the sense organs is cauterization with a hot wire or excision of the immediate nerve supply with a small iris knife. This operation, undertaken step by step, does not produce adverse changes in the fish's behavior. Regenerations of sense organs did not occur. Severing of main nerves was not done, because the sensitivity of the skin is simultaneously reduced as a result of such procedures, and this is to be avoided.

MORPHOLOGY

The lateral line of the genus *Fundulus*, in particular *F. heteroclitus*, has been described several times (Denny, 1937; Gosline, 1949; von Bergeijk and Alexander, 1962); however, some anatomical features will be stressed because they are of fundamental importance for a better understanding of the experimental results. The present study concentrates on sense organs which function in the perception of surface waves. These organs are located on the top of the

head: the sense organs of the supraorbital canal system (Fig. 1). As in almost all Cyprinodontoidea (Gosline, 1949; Rosen and Mendelson, 1960; Schwartz, unpublished data) these organs occur in groups. They are called *nasal*, *supraorbital*, and *postorbital*.

The sense organs of the supraorbital canal system of the lateral line (SLL) are located somewhat marginally on top of the strongly flattened head and are therefore in the same plane. The canals are raised slightly above the skin. If a fish hovers just beneath the water surface its organs touch the surface film and thus represent a morphological adaptation whereby as many receptors as possible are exposed to waves, which are limited chiefly to the surface itself. In this connection, it should be mentioned that only a few neuromasts, but no lateral line canal, are present on the side of the body behind the head. Denny (1937) and von Bergeijk and Alexander (1962) can be consulted for a more complete description of the lateral line organs.

FIG. 1. Arrangement of the sense organs of the lateral line on top of the head of *F. notatus:* nas — nasal; spo — supraorbital; po — postorbital canal; pto — pitorgan.

A dorsal view of the head of a fish and the configuration of the canals, pores, and free-standing organs deserve emphasis (Fig. 1). The sense organs are located on either side, symmetrically to the median line of the fish. Moreover, differences in angles between the single canals or the free organs and the median line of the fish are noteworthy and significant.

The free-standing organs (normally two), on each side of the snout somewhat medial to the nasal canal, are slightly oval-shaped. The cupula is two to three times as long as broad (0.24–0.41 mm) and represents the longitudinal axis of the sense organ, which forms an angle of 3–12° from the midline of the fish. The nasal canal is located at an angle of 8–11°, the anterior part of the supraorbital canal at 172°, the posterior one at 47–52°, and the upper part of the postorbital canal differs 106° from the midline. The geometric measurements were made as follows: a line was drawn through the center of the two pores of a canal. The angle between this line and the midline of the fish was measured under the binocular microscope or from photographs. The sensory structure in a canal appears to be an oval patch with the cupula arising along the long axis, which is parallel to the axis of the canal.

RESULTS

Adjustment to Depth of Water

Two species of the genus *Fundulus* (*notatus* and *diaphanus* Le Sueur) are native to Lake Mendota, Wisc. (McNaught 1963). Both have been described as preferring the quiet shallow water of streams, ponds, and lakes. The preference for a certain water depth was observed in the laboratory. Both species were kept separated under the same conditions in tanks (water depth, 25 cm). A series of experiments was carried out over a period of 2 months (Fig. 2),

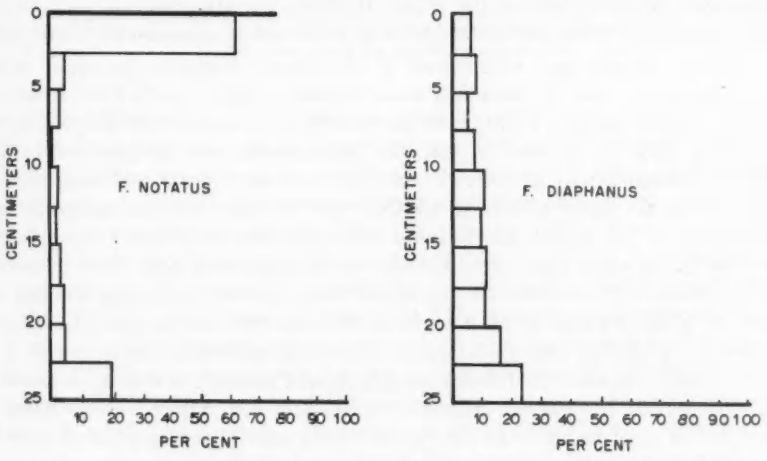

FIG. 2. Vertical distribution of *F. notatus* and *F. diaphanus* in an aquarium under equal conditions.

initially with single fish and then with 10 fish in a tank, with similar results. The observations were made at random times from 0800–2200 hr and food was offered equally at the bottom, midwater, and surface. Over this period *F. notatus* hovered in the top 2.5 cm of water 63% of the time and, in three out of four observations, was found in direct contact with the surface film. The fish were fairly homogeneously distributed by depth at other intervals except in the bottom zone, where it appeared that they were in search of food. This species achieves closer and more frequent contact with the water surface at night than during the day. If artificial lights are turned on at night, the fish drop to the bottom, where they remain for at least 2 min. An enucleated *F. notatus* hovers or moves slowly in contact with the surface film at least 94% of the time.

Fundulus diaphanus appears less commonly in the upper 2.5 cm of the water column but prefers the depths beneath this zone.

This distribution changes radically if the water surface is disturbed by aeration or stirring for then both species remain in the deeper water.

Response to Surface Waves

Observations made clear that this species quickly takes food which drops on the surface, even when the food is relatively far away and perhaps out of sight. Thus, it is obvious that not only the eye but other sense organs such as the lateral line enable these animals to detect their food.

A surprisingly high percentage of *F. notatus* (65%) reacted spontaneously to surface waves without prior introduction of food. That is, they point consistently towards the wave source. In training, this response was rewarded with food, a *Tubifex* worm presented to the fish with forceps. Food reward was offered only at the origin of the wave. If a fish did not react at once, it was trained to do so. After seven trials they achieved a score of 97% response.

Neither normal nor enucleated *F. diaphanus* occupy the upper water zone. Therefore a few of these fish were trained to swim up to the surface for food at a signal such as a knock at the frame of the aquarium. After 4 weeks of conditioning to surface waves, the experiment was discontinued. The resultant response was as follows: the fish became excited and snapped for food at several places although no food was served. The movements were disoriented. A fish could discriminate only between a stimulus approaching from the left or right side, provided the wave source was close to it (30 mm). The reactions of *F. notatus*, on the other hand, are well directed toward the source of a disturbance on the surface. Because the lateral line of the two species has a similar morphology, this result is somewhat unexpected. One might speculate about the differences in selection pressure which have resulted in a differential behavioral response in species with similar morphological adaptions. It may be that the two species have specialized in order to coexist in the same habitat.

Function of the Sense Organs of SLL

While in contact with the surface film, *F. notatus* responds to surface waves approaching from all directions. The response is composed of a quick orientation of the head in the direction of the wave source and an immediate movement towards it (Fig. 3a, b). The accuracy of such a response is greater when the wave source is located anteriorly or laterally, but less posteriorly. The surface waves travel fairly slowly from the wave source. The reaction of a fish takes place at the moment the waves strike. Again and again, trials with a wave source at various distances suggest that no other stimulus is involved in this response of the animal. Another type of observation supports this statement: the fish does not respond to surface waves if it is restricted from the surface film.

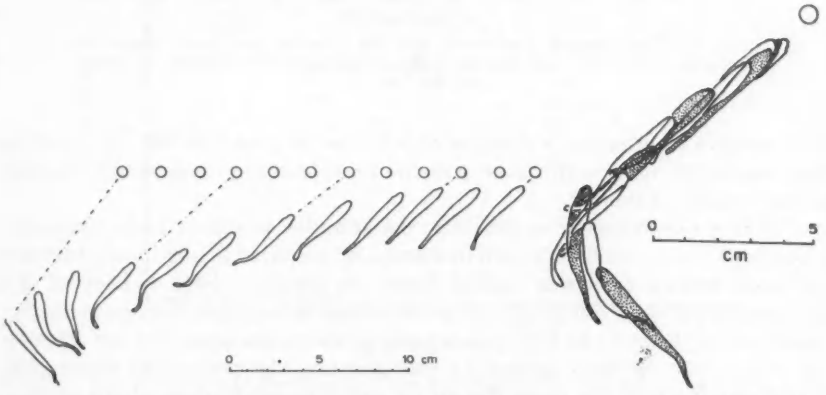

FIG. 3. (*Left*) Traces of each 3rd movie frame (24 fr/sec) show the stages of a positive reaction, and (*Right*) demonstration of the rate of forward movement of the fish during this reaction. Stippled forms are alternating positions.

Waves with low amplitudes, as used in these experiments, are mainly limited to the surface itself. These waves travel in a horizontal plane along the surface in two dimensions of the three-dimensional space. The morphologically adapted lateral line system, in contact with this plane has, it appears, an omnidirectional sensitivity to it. The perceptibility of surface waves, as a function of the distance of the wave source from the fish, is depicted in Fig. 4. At increased distances, the amplitude of the stimulus was increased by using rods of thicker glass. Care was taken that the fish was motionless at the onset of each experiment. The waves approach the fish anteriorly, that is, the wave source is never located behind a line drawn horizontally through both eyes. The unbroken curve in Fig. 4 (closed circles) represents the average score of seven fish; the open circles, those of individual fish. Up to a distance of 400 mm, the fish achieve an excellent score. This unusual perceptivity and reaction to stimuli drops steadily with increasing distance, but the fish still demonstrate

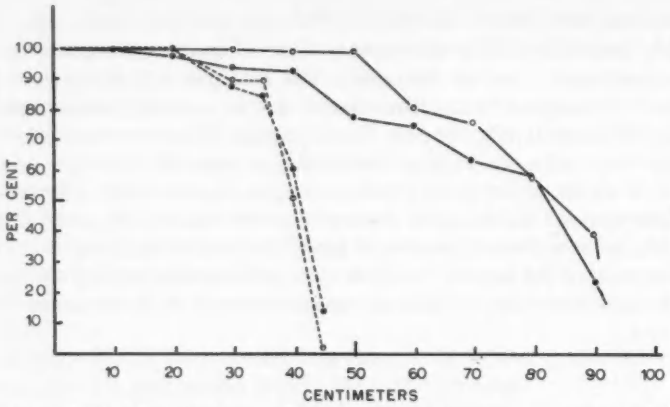

FIG. 4. The absolute (————) and the relative (----------) range of distance perception of surface waves. ●: average score of 7 animals, ○: score of fish No 7.

59% positive reactions at a distance of 800 mm. Beyond 800–900 mm, a sharp decrease to 24% occurs. However, a distinct response can frequently be elicited at even greater distances.

During the following experiment, the stimulus was kept fairly constant. A needle (1 mm in diameter) fixed in a specially prepared holder barely touched the water surface as it was pushed down. In the immediate vicinity of the wave source we again obtained positive responses throughout, but, surprisingly, a sudden drop from 84 to 13% positive responses results when fish are 350–450 mm away from the wave source. In this instance, the continuous diminution of the amplitude of the wave during propagation establishes a limit of perception, rather than some other attribute of the wave.

REACTION

It became apparent, during the first conditioning experiments, that the fish respond differently to waves generated nearby than to those at some distance away from them. Reactions caused by waves originating nearby are quick and vigorous, while the reactions to waves from a distance are slower, more balanced, and longer lasting. But even the reactions in this near field indicate that the fish is capable of estimating the distance to the wave source. This capability was tested in a great number of tests with 11 fish (Fig. 5a, b). Three of these did not show graduated forward movements and their responses did not change toward stimuli from various distances; nevertheless, a well-executed directional orientation occurred. Such a deficiency in some fish may result from a shock or some other previous disturbance or injury. Sporadically, weak reactions following strong disturbances are observed.

A reaction was considered positive when a fish stopped within 5 mm of the wave source. The movement terminated in an abrupt stop. In trained

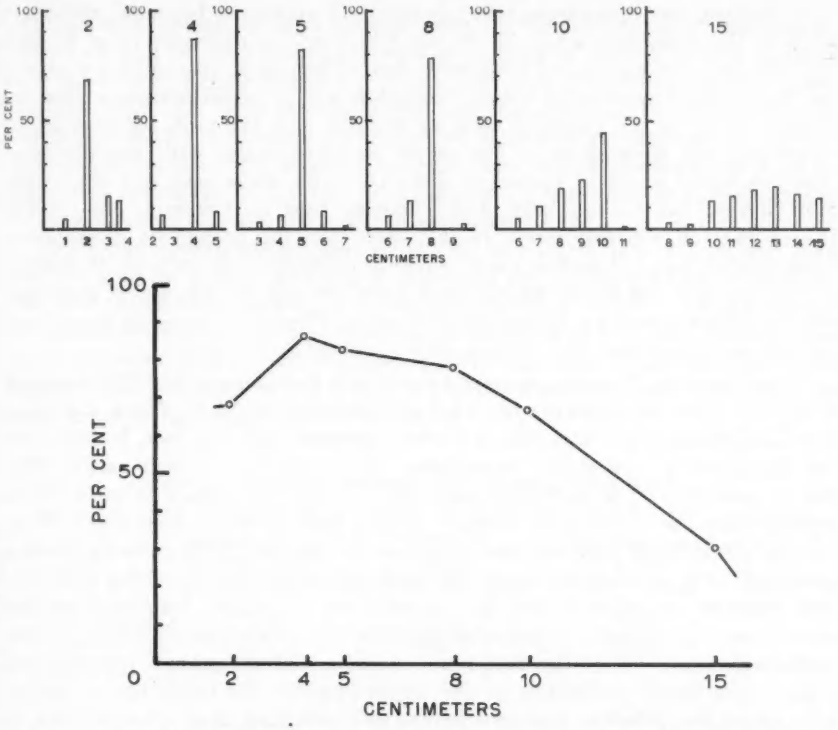

FIG. 5. (*Top*) Estimation of the distance of the wave source from the fish in the 2, 4, 5, 8, 10, and 15-cm test series. The columns represent the achieved score in per cent. (Bottom) Positive responses to the tests at distances of 0–15 cm.

fish an immediate snapping often follows the orienting reactions. Distance estimation is most precise when the wave source is 40–50 mm away from the fish. In these instances, the fish attained 86.5 and 82% positive reactions, respectively. The precision of the reactions decreases with increasing distance, from 86.5% at 40 mm to 14% at 150 mm and, correspondingly, the variation of the responses increases from 25 mm at 20 and 40 mm to 70 mm at 150 mm. One must be cognizant of the fact that a target appears to be smaller the farther it is away. Within a limited range of the target, of course, a fish can strike more easily from a distance of 50 mm than from 150 mm. In this regard, a larger target was provided for the greater distances, in order to simulate or compensate for a similar size. Therefore, it was enlarged to ± 8 mm at 80 mm, ± 10 mm at 100 mm, and ± 15 mm at 150 mm, and it was decreased to ± 2 mm at 20 mm. Actually, the precision of the reactions decreases on this basis from 86.5% at 40 mm to 30.3%, instead of 14% at 150 mm, and the variation increases from 25 mm at 40 to 55 mm at 150 mm tests series.

Another fact concerning the variation of reactions becomes apparent: 28% of the experiments for the 20 mm test result in reactions 10 or 15 mm beyond the wave source; only 4% were short. The test at 40 and 50 mm shows an almost even number of misses (overshooting or undershooting). One recognizes that in the following tests at 80, 100, and 150 mm, the number of reactions that were short of the target becomes larger with the distance. The fish failed to travel the entire distance to the wave source, 7.8% of the time during the 80 mm test, 33.6% during that at 100 mm, and 69.7% during the 150 mm test series. In the same way, the proportion of reactions from farther away than the wave source diminished from 28% at 20 mm to 2.5% at 80 mm and finally disappeared in the 100 and 150 mm series. *Fundulus notatus* does not reach a target 200 mm away. The reactions occur sometimes as close as 30 mm but vary considerably, from 80 to 170 mm.

In summary, *F. notatus* responds sensitively to surface waves. The response is directed towards the wave source and is as accurate as $\pm 1.5°$. Waves traveling over a distance of 950 mm still produce a response from the fish. In addition, the fish develops an ability to estimate distances over a short range. This raises questions: How are these fish able to orient to surface waves? What enables them to distinguish between stimuli from different distances? What are the stimuli and how are they analyzed by the fish? The radiating waves generated at a point become more and more expanded with increasing distance. The front of the wave is strongly curved near its source, but becomes less curved as it progresses; correspondingly, the rate of change of the arc of the wave is greatest within the first few centimeters of the wave's propagation. This characteristic difference of the dimensions of the curvature of waves may cause the different reactions of the fish assuming that a fish is able to recognize these changes. The fish respond differentially to waves originating from a point less than 150 mm away, where the greatest change of the dimensions of the curvature of the waves occurs. A wave, however, produced by dropping a ring of plexiglass (75 mm diameter) onto the water evokes a weaker reaction than those responses which are typical for waves produced by a point source at a similar distance. Here the curvature of the wave is less steep. Often, no responses were obtained under such conditions although, before and after such a test, the fish responded regularly. In this instance, the wave source must seem to the fish to be farther away than it actually is. This observation directs attention to the role of the curvature of the surface waves and the arrangement of the perceiving sense organs on the head.

Reaction Time

As stated before, a response to surface waves occurs at the moment the waves strike the fish. In order to determine the time necessary to release a reaction after the first wave front arrived at the fish, a moving picture film was made. The camera was fixed directly above the experimental tank. Therefore the picture presents true distance proportions. The waves are pictured

as concentric circles. A light shining from the side illuminated the experimental tank. This adjustment provided a contrasting picture of disturbances of the surface. The waves show bright and dark borders depending upon the location of the light source. Waves are best observed between the wave source and the light. However, a shadow of these surface waves at the bottom of the tank could not be avoided. A single frame movie projector was used to analyze the color film. Color film (Kodachrome II) was used for photographing 14 experiments. The approach of the waves could easily be seen on the film as could the propagation speed of the waves, which is 816 mm/sec. The reaction time as defined above was measured by counting frames (Table I). The film speed was verified in a control experiment with a timer used for timing spark frequency of gasoline engines (accurate to 0.02 sec). The shortest time between stimulus and reaction in two experiments was 0.038 sec; the longest, 0.067 sec, with an average of 0.05 sec. A time interval of this duration is sufficient to release an oriented response.

TABLE I. Reaction time determined through motion pictures.

Number of experiments	Frames 64/sec	Reaction time (*sec*)
2	3	0.036
5	after 3	0.046
4	4	0.053
2	after 4	0.060
1	5	0.067
Average: 14	after 3	0.051

RESPONSES TO TWO WAVES

The fact that these fish could be guided to take up desired places in the tank facilitated this experiment considerably. The stimulus is composed of two surface waves of equal strength originating simultaneously (see Methods). The waves progressing from the two origins create identical wave fields to the right and left of their median line. A fish located in the right field, for instance, will receive waves first from the right hand source. Consequently it responds by orienting to this origin. A reaction to the closer of the two wave sources takes place independently of the location and position of the fish. A response from a position which is only a few millimeters away from the median line is influenced somewhat by the wave from the other side. Initially, the fish hesitates but then moves to the less distant wave source.

The fish reacts differently when it lies exactly on the median line of the two wave sources. That is, there was no indication that the fish could differentiate between the two sources, because the fish advances along this median

line. Quite often a fish deviates to the right or left of the median line during its movement and moves immediately to the closer source. A fish lying on the median line, but close to the wave sources, reacts weakly and briefly, but if it is farther away, a stronger and longer-lasting response is elicited. Two of eight fish showed almost a constant reaction regardless of the distance. All reactions on the median line are shorter and slower than those towards a single wave source. Almost no reaction was obtained at the intersection of the connecting and median line. If a fish swims through this point when waves are presented, it stops at once. The behavior was characterized by the fish moving its head back and forth, bending the trunk, or by an increased beating of its pectoral fins.

In a situation in which the time difference of arrival of the two waves is sufficiently great, the fish correct their response. A fish, placed somewhat behind a wave source near the median line, responds first to the wave from the closer wave source and moves towards it, whereas if the wave from the second source approaches, another response is made. Fish always react to the first wave regardless of whether the fish is closer to one wave source, or whether one point of the fork pricks the water earlier than the other.

In summary, the smaller the angle between the fish's mouth and the two wave sources, the more vigorous the response. A fish does not distinguish between identical stimuli, but moves forward along the median line. It distinguishes, however, between situations where a time or an intensity difference is involved. In order to explain these results, we suggest that the distribution of the sense organs on the head is a functional one and that the sense organs themselves function differently. By this we mean that certain sense organs are sensitive to stimuli occurring anteriorly and others laterally, but not vice versa. It follows from this assumption, then, that the desired response occurs as a result of stimulation of the proper organs.

If a fish is located exactly between the origin of two sources, the waves will arrive at the same time and with the same strength, but, because they are opposed in their directions, they appear to neutralize each other and have no effect. If the angle between the two wave sources and the fish's mouth, called here α, becomes smaller, that is, if the wave sources move in front of the fish, these antagonizing stimuli become less meaningful but not less effective. Other sense organs, previously unstimulated now perceive waves, while the former are less stimulated. This change of the effective stimuli increases with the decrease in the angle α or, the closer the two wave sources are together or, the farther the fish is away from them (up to 150 mm). Such a model is consistent with the results of the tests, which might otherwise appear contradictory.

Partial and Total Elimination of Sense Organs

The results of the experiment with two simultaneous surface waves raise questions concerning the functioning of individual organs, their significance for perception of a stimulus, and the interaction of these organs. Behavioral

studies have not yet demonstrated the distinct perception of the direction of a water current. Dijkgraaf (1934) stimulated a certain part of the lateral line of *Phoxinus laevis* with a water current from different directions and the responses remained the same. He concluded that a discrimination of the direction of the water current is not made. Similarly, Kramer (1953) observed that a group of individual sense organs in *Xenopus laevis* is represented by a "starres Lokalzeichen" which does not imply differentiation of the direction of the stimulus. On the other hand, by electrophysiological methods, Sand (1937) recorded an increase or an inhibition of the nerve fiber activity depending on the direction of the water flow within a lateral line canal of *Raja*. Likewise Dijkgraaf (1956) and Görner (1963) demonstrated that an individual sense organ of the lateral line in *Xenopus laevis* has a bidirectional sensitivity.

The surface fish *F. notatus* fulfils some of the requirements necessary for an experiment on the function of individual sense organs and their interactions. Favorable conditions for perception and differentiation result from the ideal location of the organs. Also conducive to experimentation are the short canals containing single sense organs, well separated from one another, and to the behavioral preference of the fish to stay in contact with the water surface where it can receive surface waves. The location of the wave source and the position of the fish are readily determined and related to each other, because the factor of water depth is eliminated. The wave sources, the wave, and the receptors of the fish are on the same plane, i.e. the water surface.

After the elimination of the sense organs of the supraorbital canal system, the fish no longer respond to surface waves. A strong surface wave produced nearby, however, induces a weak reaction. The fish also snap repeatedly if they are touched with food indicating that the sensitivity of the skin is intact. Elimination of lateral line sense organs does not appear to change the fish's behavior. In summary, *F. notatus* perceives surface waves by means of the supraorbital canal system (SLL).

In order to study the interaction of individual sense organs, several combinations of functional organs were tested. Enucleated fish used in the following experiments were examined for their ability to respond to surface waves before sense organs were eliminated in order to determine the consequences of deprival of sense organs. Therefore, only those animals which showed a strong response were employed. The procedure of removing the organs has been previously described. Experiments began as soon as an operated fish fed regularly.

ELIMINATION OF THE SENSE ORGANS ON ONE SIDE OF THE HEAD

Waves approaching a fish at right angles to the operated side do not produce a response. Occasionally, however, a reaction away from the wave source was demonstrated, a result at variance with the usual case.

A wave which approaches the intact side of a fish always releases a reaction directed approximately towards the wave source. The operated fish snap

for food often unsuccessfully, while before the operation they seldom miss. When operated they missed the food owing to deviations toward the intact side.

When the waves were generated in front of the fish, the distance of the wave source from the fish varied from 10 to 150 mm. If the sense organs of the left side are removed, the fish will deviate to the right, and vice versa. The degree of this deviation seems to be relatively constant regardless of the distance of the wave source. The average value is 42° and the extreme values range from 35 to 55°. These responses still reveal some capability for graded reactions, although of shorter duration than normally. By increasing the number of stimuli (waves) per unit time the degree of deviation becomes more extreme (Fig. 6). The angle of deviation at the beginning of the movement was 50–60° and increased steadily to 85–90°, toward the end. The fish regularly moved

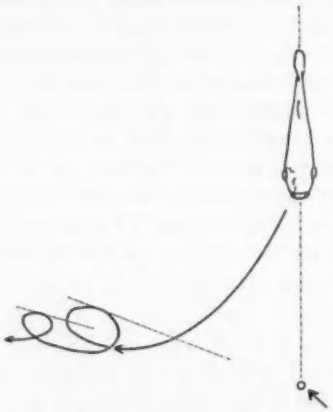

FIG. 6. Example of a fish, whose sense organs are removed from one side of the head, where it responds to a continuous stimulus with deviations towards the intact side.

away from the wave source, but quite often the reaction caused by a continuous stimulus did not end this way. That is, the fish attained an angle of 95° or a little more. The intact side of the animal was now suddenly exposed again to the waves. Consequently the fish turned, moved towards the wave source and, simultaneously, the deviation occurred. The fish moved in an ellipse. The following traverse, however, was farther away from the wave source. A maximum of three succeeding revolutions could be obtained.

If the wave source is located in front of the fish at an angle of 30° from the intact side, the fish reacts almost normally. Waves, approaching the operated side of the fish at 20–70°, cause the fish to snap or to move forward.

A fish, whose organs were extirpated on one side of the head, always responded to waves presented in front of it but it deviated to the intact side; if the sense organs are removed from the right side, the fish moves to the left,

and vice versa. The sense organs on both sides must be stimulated equally in order to evoke a movement directly forward. Differential stimulation causes varying directed responses.

REMOVAL OF EITHER THE NASAL, SUPRAORBITAL, OR POSTORBITAL CANAL

The elimination of any of these canals influences the response considerably. First of all, we consider only the directed response: removal of either the nasal or the supraorbital canal indicates that the capability of a fish to react in a well-oriented manner is not substantially attenuated. Operated fish are able to make nearly directed responses with the precision of an intact fish. The removal of the postorbital canal, however, produces a big gap in the omnidirectional reception field. This gap was examined by moving the wave source in steps of 5° around the fish. This "blind" section is situated posteriorly and extends at an average angle of 40° from the fish's midline. The center point of these measurements is the intersection of the midline and a line drawn through the center of both eyes. The directed reactions in the receptional field became somewhat unstable.

These experiments reveal, in addition, a significant defect in distance estimation: the elimination of the postorbital canal has very little effect, while elimination of either the nasal or supraorbital canal has a greater effect. The reactions are characterized by being slower and weaker than those of normal animals. The extent of forward movement varies with the individual, but the reactions are always too short, and even shorter if the nasal or supraorbital canal is removed. In addition, mistakes in snapping for food occur frequently, four out of five compared to one out of ten for a normal animal. These results again emphasize the instability of those reactions.

Summarizing from these experiments, it is clear that the elimination of either the nasal or supraorbital canal impairs the accuracy for estimating distance. Furthermore, after removal of the postorbital canal a "blind" section behind the fish appears in its receptional field. Finally, it can be assumed that, because no "blind" area could be detected following the removal of either the nasal or the supraorbital canal, one of them appears to substitute for the other.

Perceptional Field of Individual and Groups of Sense Organs

A reduced set of sense organs, it is assumed, transmits less "information" to the brain than the normal set. This causes hesitation as well as a weaker response. These responses, however, were distinct enough to perform the following experiments.

NASAL CANAL

All sense organs of the SLL were removed except the nasal canal on one side of the head. The experiments were performed with individual organs situated both to the right and to the left of the midline. The nasal canal is placed at an angle of 15° to the midline of the fish. The remaining perceptional field is a section of about 85° moved slightly toward the side and in front of

the fish, extending from −20° to +65° (Fig. 7). When the wave source was restricted to approximately +20°, the fish responded most vigorously. The reactions are fairly well directed and immediate, but much too short. The fish advance 30–40 mm when a wave is generated at a distance of 80 mm. The accuracy of this remarkable, well-oriented reaction diminishes when the wave source is moved to the right or left. The fish now detect only the approximate direction of the wave source. Near the margin of the perceptional field, a faint reaction is barely observed. Thus, a single functional nasal canal organ limits the fish's perceptional field considerably and restricts its ability to make a precise response.

Only the two free-standing organs (neuromasts) anterior to the nasal canal now remain functional. A ridge, the cupula, rises from these oval organs along the longitudinal axis. Their function was examined in several instances.

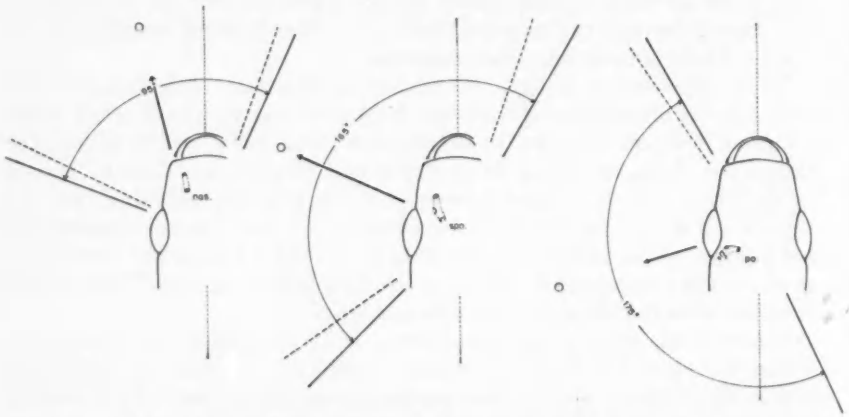

FIG. 7. The perceptional field of individual sense organs: *left*: nasal; *middle*: supraorbital; *right*: postorbital canal. The arrows represent the relative strength of the responses of the fish in the most sensitive area of the field proper.

Only those waves originating from a small section of the original field caused a reaction. This section extends from 5 to 40° on the left side, if the organs on the left are functional, and vice versa. The reaction itself is faint and short of the target.

The section of the remaining perceptional field of the operated fish is located around the extension of the longitudinal axis of the sense organ, which is also the longitudinal axis of the cupula. Principally, the same situation obtains for the nasal canal. The field of perception is immediately anterior to the canal. Thus, an anterior stimulus is perceived, but not a lateral one.

SUPRAORBITAL CANAL

This canal involves two sense organs. One pore of the canal opens anterior to the nare; another, lateral; and a third, posterior (Fig. 7). The canal is

slightly curved, the anterior part being, therefore, at a lesser angle from the midline of the fish than the posterior one. Such an arrangement covers a major part of the perceptional field of an intact fish. The section extends from −20° to +125°, a total of 145°, in five fish. Extreme values, from −40° to +130° are represented by animal F_6. A wave approaching from a segment of 50–90° elicited the most definite response from these animals. After a relatively short movement, the oriented reaction terminates, often with snapping. Stimuli originating in the anterior or posterior area of the perceptional field produce reactions which are directed approximately to the intermediate area so that the fish mistake the direction in both cases. Also, a stimulus presented exactly in front of the fish results in a deviation of 30–35° (extreme, 50°) toward the intact side of the fish from an axis extending from the fish to the wave source. A faint response due to a wave originating between 0 and −20° towards the area of origin of the stimulus is observed in all animals. A turn approximately towards the wave source, however, no longer occurs beyond an angle of 105°. In the latter case, the fish moves somewhat to the side of the stimulus, but away from the wave source.

In summary, it follows that the perceptional fields of the supraorbital and the nasal canal overlap each other. That of the first one covers nearly the whole field of the nasal canal. However, the supraorbital canal is most sensitive to waves approaching the side of the fish, while the nasal canal is most sensitive to those from ahead.

POSTORBITAL CANAL

Three sense organs form this part of the system. Sometimes they are free-standing organs. The oval patches with the projecting cupula along the longitudinal axis can easily be seen. Sometimes they are enclosed in a short, but bent canal. The first sensory patch encompasses an angle of 80°, the second 130°, and the third — not really enclosed but just covered by a roof of skin — about 5° from the fish's midline. Both types were examined and no functional differences were detected. The remaining perceptional field, following extirpation, extends from 35° through 180 to −155°. The area of most precise response ranges from 55 to 160°. The responses of the fish elicited from waves originating at both ends of the perceptional field are weaker. The reactions are highly stereotyped, even at varying distances, and cease after a very short time. It should be added that a response results also from a stimulus which is presented just in front of a fish. In one out of every six tests, the fish respond to such a wave by swimming jerkily forward about 2–3 cm regardless of how distant the wave source. Displacement of the wave source revealed that this latter response is just a forward movement and not an oriented reaction.

In recapitulation, it appears established that the sense organs of the postorbital canal are arranged effectively to receive surface waves laterally and posteriorly, but not anteriorly.

The role of sense organs in recording a stimulus in the omnidirectional perceptional field of the fish is considered briefly. Each of these three canals is

particularly sensitive to waves approaching the fish from a small specific section. Thus, the nasal canal is mainly oriented anteriorly, the supraorbital canal laterally and slightly anteriorly; and the postorbital canal posteriorly. These canals also represent, according to their number of sensory patches, different large sections of the original perceptional field; for instance, the only organ of the nasal canal monitors a section of 85°; the two supraorbital organs, 145°; and the three postorbital canal organs, 170°. The perceptional fields of these canals overlap one another (Fig. 8). This coverage of a con-

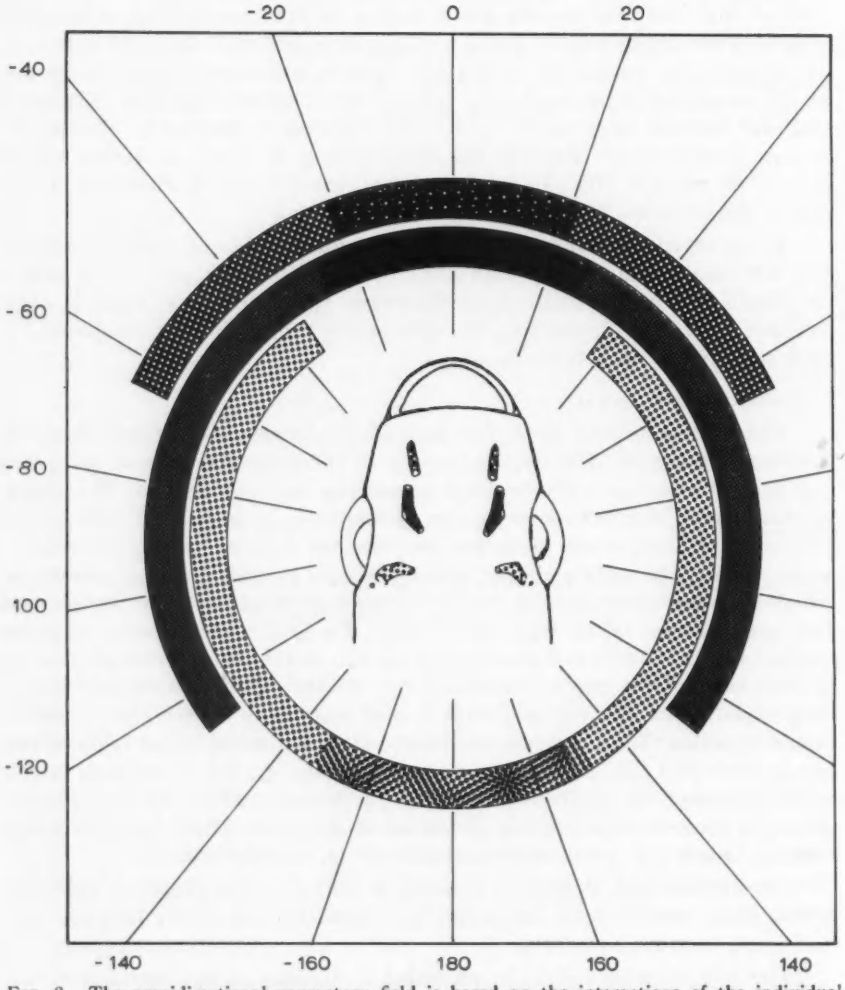

FIG. 8. The omnidirectional perceptory field is based on the interactions of the individual sense organs: *upper* — nasal; *centre* — supraorbital; *lower* — postorbital.

siderable part of one perceptional field by another one seems to be important for the precise response. The most frequent overlap of these fields occurs in front of the fish. A wave from this area, therefore, stimulates at least 10 individual sense organs and makes possible an accurate localization.

Vision and Distant Touch

Laboratory and field observations emphasize the dominant role of the eye in the life of these fish. This is expressed not only in a tendency for fright reactions when a shadow passes over the water, but also by a readiness to examine a small moving object, possibly a prey. These objects may be water drops running down the outside of an aquarium or a small glass rod moving in front of the tank. The fish have excellent vision in water. But, because *F. notatus* lives very close to the water surface, the "aerial window" (Walls, 1942) and hence the upward visual field is much smaller than in fish frequenting the deeper water. The "aerial window" is limited by the refraction and reflection of light at the water–air interface. A total reflection occurs when the angle is less than 48.8°. But, in the marginal parts of the theoretical upward visual field, there occurs a strong refraction, which is responsible for an appreciable reduction in size of objects on the periphery of the "window," a refraction of the light into its spectrum, and a partial reflection, or mirror effect. Seeing objects out of water along the water surface is indeed almost impossible. In one experiment, food lying upon a glass plate, about 10 mm above the surface, was offered to hungry fish. The animal, being close to the surface, discovered the food when it was 40–60 mm away. At first the fish are startled by the food then swim just beneath it and jump out of the water to reach it. On the other hand, a fish, not in contact with the surface, but near it, discovers food at a distance of 200 mm only if the food sinks into the water. The fish show no response to food that continues to float on the surface. An almost blind band between the downward and upward visual field exists at the water–air interface. Nevertheless, a fish, in contact with the surface, is able to find its prey very well. Hence, we conclude that the lateral line supplements the vision of this species.

SIGNIFICANCE

Both morphological and functional adaptation for a life at the surface film are represented well in *F. notatus*. Perception of surface waves by the sense organs of the lateral line system enables these fish to locate prey on the water surface at a great distance, at least up to 900 mm, with high scores.

The experiments demonstrate that a stimulus striking parallel to the flag-like cupula of a free-standing sense organ, or parallel to a canal organ evokes a response of the animal, but no response is elicited from those approaching perpendicularly. The effective stimulus apparently causes a gliding movement of the cupula along the macula, and the resultant shearing force stimulates the sensory cells. This view is in accordance with the functioning of other types of sense organs of the acoustico-lateralis system.

Individual Organs

The directional property of individual sense organs and, hence, their arrangement on the head of the fish, is of profound importance. An individual sense organ functions, first, selectively and, secondly, as an analyzing receptor. The fish react to stimuli received from a limited area with an oriented move toward the origin of the wave. Consequently, through interactions of these organs, the omnidirectional sensitivity is established, and well-directed responses are made for all locations of a stimulus. Organs located symmetrically are of importance for well-oriented reactions as is revealed by rendering the organs nonfunctional. Removal of sense organs from one side of the head causes only disorientation. It appears that each sense organ, depending on its location, delivers impulses which have specific meaning and which are modified according to the direction from which the stimulus originates. This "information" obtained from an individual organ then is compared, for example, with that from the symmetrically located organ on the other side of the body. The result of this resolution or comparison is the directed response. The estimation of distance is due to the close interaction of sense organs in sequence whose fields are directed at different angles from the midline of the fish — the nasal and supraorbital organs. They are morphologically separated, but function as a unit. These individual sense organs perceive a stimulus of a specific strength, which depends on their configuration. The degree of stimulation of one sense organ, obviously, is strictly related to that of another. By varying the distance of the wave source from the fish, one changes simultaneously the curvature of the waves and, therefore, the direction and timing of the stimulus. This again changes the degree of stimulation of the organs. Consequently, the fish obtain cues about the distance to the point source of the wave. The experiments demonstrate that, over short ranges of about 100–150 mm, the fish estimates distances correctly.

Stimulus to the Sense Organ

The lateral line organs are sensitive to surface waves. Such waves differ from sound waves because of their different physical properties. Although not sine waves, surface waves — at very low intensities — are approximately sinusoidal. The velocity of progressive waves is not constant, but depends on the frequency. That the sensitivity of the lateral line of *Aplocheilus lineatus* depends on the frequency was demonstrated by Schwartz (1965). The water particles oscillate normally to the direction of travel of the waves. They actually move in small circles in dissipating energy. Hence, surface waves are characterized by narrow crests and wide antinodes. A preliminary calculation revealed that the displacement of water particles in surface waves can be considerably above the threshold for cupula organs. The threshold for sense organs of the lateral line owing to damming phenomena has been roughly determined by Harris and von Bergeijk (1962) at "some $10^{-1}\mu$", and by Kuiper (1956) at about $10^{-3}\mu$ in the ruff (*Acerina*) and Groen estimated the threshold for the cupula organ in the ampullae of the semicircular canals in human beings at $10^{-1}\mu$

(cited from Dijkgraaf, 1963). Corresponding values for propagated sound waves, estimated at about $10^{-9}\mu$ by Pumphry (1950), still remain distinctly below these figures. We plan to undertake a study to determine the threshold for the organ under such conditions.

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Changes in Chemical Composition of Central British Columbia Pink Salmon During Early Sea Life[1]

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ABSTRACT

Changes taking place in body composition of juvenile pink salmon during early sea life were studied. Under the natural conditions obtained in 1963 and 1964 the fish became relatively more obese as they grew to a length of 7.5 cm. Further growth in weight was isometric with growth in length. Water progressively became a lesser proportion of total weight until 7.5 cm length, thereafter it remained an approximately constant proportion. A diurnal fluctuation in body moisture was noted, and was correlated with a diurnal feeding cycle. Protein, in respect to length, showed positive allometry throughout the size range studied but an inflection in the allometric line was noted at about 5.5 cm. Relative lipid weight is postulated to be basically correlated with protein but reflected the longer-term changes in amount of food ingested. A shift from high to low lipid content occurred at the end of May. This shift was independent of food ingested, independent of size *per se*, and occurred at the beginning of a migratory stage. Experimentally, the first reaction to severely restricted food supply was loss of lipid and moisture. Protein was catabolically consumed under more prolonged periods of starvation. At less severe conditions, lipid was lost but the body weight was maintained by uptake of water.

INTRODUCTION

Changes in the chemical composition of salmonids during ontogenesis have not received a great amount of study. In 1934, Lovern reported, for the Atlantic salmon (*Salmo salar*), a lower total lipid content in smolts than in parr. He attached no particular significance to this observation, however, as different food and growth rates might well have affected the lipid content. Furthermore, he was comparing lipid content of entire parr with that of eviscerated smolts. Hoar (1939) described an abrupt decrease in the "coefficient of condition"[2] of Atlantic salmon at smolting. This drop in the coefficient of condition occurred while the smolts were feeding voraciously, and gross dissections revealed the disappearance of visceral fat deposits prominent in presmolt parr.

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[2]Based on the assumption of isometry in the length–weight relationship.

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From studies on the chemical composition of sockeye salmon (*Oncorhynchus nerka*), Kizevetter (1948) has shown that when this species begins to feed the body is composed of about 80% moisture, 16% protein, 2% lipid, and 1% ash. With growth the percentage of moisture decreases, as does the percentage of protein, while the percentage of lipid increases threefold. At the onset of smolting the percentage of lipid decreases to less than two-thirds of the former level, but with further feeding and growth in the sea lipid increases to become 6–9% of the total body weight. Kizevetter suggested that there may be some connection between fat metabolism and the smolting migration impulse.

Corti (1950), reporting on studies of artificially reared rainbow trout (*Salmo gairdneri*), stated that at 37 days after hatching body moisture amounted to approximately 90% of total body weight. After that age body moisture became progressively a smaller portion until at 150–300 days it amounted to about 75% of the total body weight. Protein (as calculated by multiplying total nitrogen by 6.25) became a greater proportion of total body weight throughout development. From a low of 11.3% at 37 days after hatching, protein became 17.6% of total body weight at 303 days. Lipids, from a low of 2.0% at 37 days, increased to 3.3% at 70 days. At 84 days lipid had decreased to 1.7% but then increased to 7.0% at 125 days and thereafter fluctuated between 6 and 7% until the final observation of 4.5% at 303 days. The decrease in body lipids after 70 days coincided with a change in the artificial diet fed to these fish and cannot, with certainty, be considered a feature of ontogenesis.

The early life stages of central British Columbia pink salmon (*O. gorbuscha*) are in many respects similar to those of other anadromous salmonids although dissimilar environments, sizes, and time-periods are involved. Pink salmon fry, generally with little or no feeding, enter the estuaries of the natal stream shortly after emerging from the gravel. Initially, these fish are shore-oriented but, with growth, move away from the beach zone and occupy the surface waters of the enclosed bays and channels. At the close of about 6 weeks of marine growth an active migration occurs, in which the schools move seaward into the more offshore waters of the open ocean.

From the results of others, outlined above, it might be expected that changes in chemical composition of pink salmon also occur during this period of early sea life. It is of interest to determine if this is so and to compare the results with those for other species of salmonids. It is of further interest to assess which changes in body composition may be related to ontogenesis and which simply reflect fluctuations occurring in the opportunities for growth.

The opportunity for such a study came in conjunction with studies on growth and mortality of juvenile pink salmon of the Bella Coola River, central British Columbia, during the spring months of 1963 and 1964.

METHODS

Field Treatment

Unfed fry enter the Bella Coola River, British Columbia, estuary (Fig. 1) from March until early May, however, the largest numbers are seen during

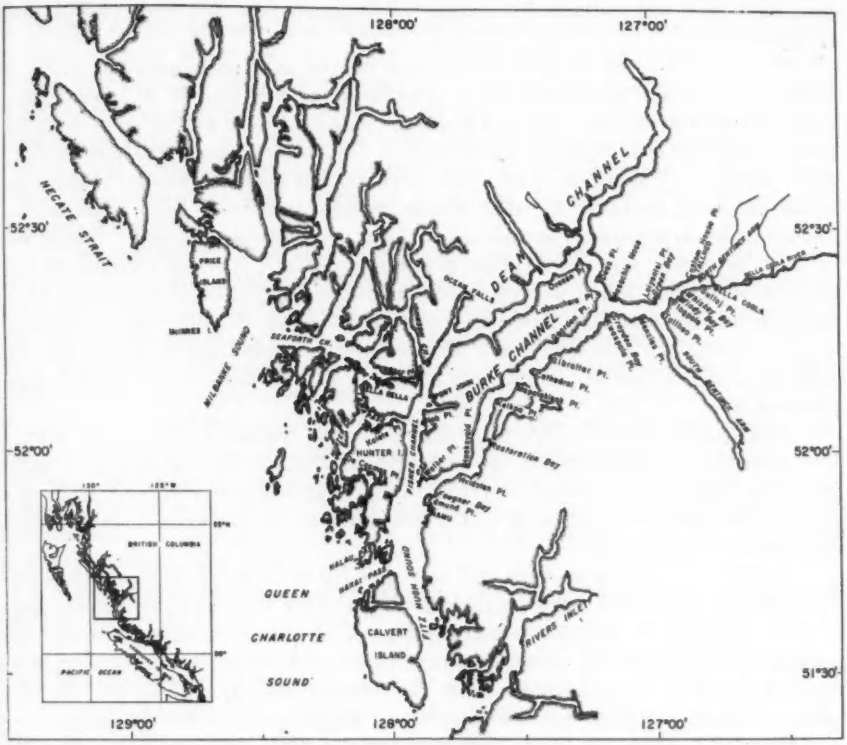

FIG. 1. A map of the central area of the British Columbia coast.

the latter half of April. The population generally moves seaward through Burke Channel and enters Fitz Hugh Sound in late May. By mid June very few juvenile pink salmon remain in the enclosed waters. During this period of 8 weeks in 1963 and 1964 samples were obtained from concentrations of juvenile pink salmon along this route using a fine-mesh purse seine. Effort was directed in the fishing to obtain the samples from the main population and it is believed that these samples are representative of and typify the Bella Coola River stock.

Following capture, the fish were transported alive to an anchored laboratory and placed in floating live cages. The ensuing treatment differed between the years.

In 1963, 100 fish were taken (without known bias) from each of 18 catches and processed immediately. Each fish was routinely anaesthetized, measured, damp-dried, and weighed according to procedures previously given (Parker, 1963). After weighing, each fish was subjectively classified into one of five relative size categories and dropped into an appropriate polyethylene bag. When the sample was completed, each bag containing 20 fish of relatively

the same size was collapsed in water to evacuate the air, then sealed and frozen.

In addition to the above observations on the wild population, a series of measurements was obtained from a population of fish held in captivity. Approximately 10,000 fish from the catch of April 21 were placed in a floating pen having the approximate dimensions of one meter cubed. The screen size, while sufficiently small to confine the fry, did not exclude plankton items of a size normally found in the diet of similar-sized free-living fish. Thus, under the imposed conditions, the amount of food available was severely restricted through crowding but not entirely eliminated. After 7 days of confinement a sample of 100 fish was processed. The remaining population was reduced to a few fish in excess of 200. After an additional 9 days of confinement another sample of 100 fish was processed. The remaining population was held for an additional 4-day period, at the end of which a final sample of 100 fish was processed.

The frozen samples were transported to the Nanaimo laboratory where they were partially thawed and the stomachs removed. Stomachs (and contents) were then weighed as subsample totals rather than individually. The gasterectomized samples were refrozen and transported to the Vancouver laboratory for proximate analysis.

In 1964, catches were held in live cages for 6–12 hr prior to processing. This holding period was sufficient to greatly reduce the amount of food contained in the stomachs. In addition, small samples of 25 fish each were preserved in seawater formalin at the time of capture to provide estimates of relative stomach weights for these catches. After the holding period, samples of 100 fish were routinely processed for live length and weight data before freezing.

From the catch of April 15 approximately 2000 fish were placed in a 60-U.S. gal (227-litre) aquarium and held without feeding until May 5. Samples of 100 fish each were periodically withdrawn from this population and processed. After 10 days mortality was evident and cannibalism could not be prevented, thus, complete starvation during the latter portion of the period was not achieved. Results during the latter portion are representative only of the survivors.

The 1964 samples were not classified into relative size-groups but were sealed entire in polyethylene bags and frozen. Stomachs were not removed, rather the frozen samples were transported directly to the Vancouver laboratory.

Biochemical Treatment

Upon receipt at Vancouver the frozen samples were pulverized in liquid nitrogen employing a mortar and pestle. Pulverizing of each sample, which was done in a cold-room at 2 C, was completed within 2 min and the frozen, powdered, samples were placed in plastic bags and stored at −20 C until analyzed. To minimize further manipulation all chemical analyses of each sample were initiated at the same time. For the analyses of 1963, duplicate portions of each sample were dried to constant weight at 105 C, the difference in initial

and final weight of the aliquot being the basis for computing total moisture. Other portions were used for total nitrogen determination by the micro-Kjeldahl procedure and a further portion was used for determination of total lipid by the procedure of Folch et al. (1957) using water as the washing medium. Triplicate portions of each sample were used for the total nitrogen determination. Crude protein, as reported, is the value for total nitrogen × 6.25. Total lipid determinations were made on duplicate aliquots of the chloroform–methanol extracts.

The samples of 1964 were pulverized and stored as above. Moisture, total nitrogen, and lipid were determined by approved methods (Association of Official Agricultural Chemists, 1960), nitrogen and lipid being determined on the dry residue remaining from the moisture determination.[3]

While the estimates of relative moisture and protein are directly comparable between the 2 years, the change in lipid analysis in 1964 led to a lower extraction of lipid material. A comparison of the two methods was carried out on an aliquot from one of the samples of 1964 which revealed that the 1963 method estimated approximately 2.6 times the lipid of the method used in 1964.[4] This value is assumed to be applicable as a correction coefficient for all 1964 determinations and is applied to those data to allow comparison of lipid contents between the years.

Results of these basic measurements and determinations are presented in Appendices A–D.

Statistical Treatment

A comparison of the measurements given in Appendices A and B reveals four general features. As the season progressed and/or the size of the average individual increased, (1) the percentage of body moisture decreased, (2) the percentage of protein increased, (3) the percentage of lipid increased initially but subsequently fell, and (4) a rise and fall similar to that of lipids occurred within the relative stomach weights. An answer to the question of environmentally vs. genetically induced change in body composition is not readily apparent from the data in its present form. This difficulty arises from two sources: (a) With the samples arrayed in chronological order, there is confusion among the actual ages of the fish as measured from time of entrance into the sea. Since the time of entrance into the sea extends over more than 4 weeks, fish in any single sample are of a variety of sizes and ages. In this analysis size will be taken as the best indicator of marine age and the samples will be arrayed according to the average length statistics of the sample. (b) Difficulty arises in detecting changes in body composition when the data are in a form of percentage of total weight. For instance, a fish may gain water, which is then reflected in a proportionate decrease in protein and lipids, although there may

[3]The samples of 1964 were analyzed by Coast Eldridge Engineers and Chemists Limited, Vancouver, B.C.

[4]From this experience and that of others, it is apparent that it is very difficult to extract total lipid from dried fish products.

have been no actual loss or even cessation of the increase of these constituents. Thus, it is necessary to estimate the actual weights of each body component. As a fish grows, however, the weights of these components increase, hence it is necessary to estimate the weight of each component for a fish of standard size, assuming no alteration in relative weight or shape.

We have utilized the well-known allometric equation,

$$w = al^b$$

where w denotes weight, l denotes length, a and b being unknowns. The exact determination of a and b is not necessary to this presentation. Instead, arguments and conclusions will be advanced on the basis of geometrical presentations. In log form, the allometric equation is linear. The exponent b is identified as the slope of average regression of log w on log l, and log a is the intercept or the height of the regression line where it crosses the 0 ordinate. Thus, a is identified as the average weight where $l = 1$ cm and corresponding values can be calculated for any other selected value of l.

Comparison of geometric plots of data having apparent regression slopes close to 3 are difficult to judge "by eye." Such data may be modified to bring the apparent slopes to approximately 0, in which case deviations from the horizontal axis of all or part of the plots are more easily detected. The various values of body weight and percentages of composition given in Appendices A and C have been modified to estimate weights at a standard length of 4 cm using $b = 3$. These standardized values are given in Tables I and II and a sample calculation of the adjusted value is given in Table I (footnotes a and b). Thus, the first row in Table I shows the estimated weights of the body and each of its constituents for the average fish of sample 1a adjusted to a length of 4.0 cm and assuming no change in relative proportions. This transformation has the effect of removing 3 from the exponent of the allometric equation.

Sources of Error

Original lengths were accurate to ± 1 mm. Original weights, estimated to the nearest 0.01 g, are considered accurate to within $\pm 2\%$ (Parker, 1963). Average lengths and weights given in Appendices A–D may be considered significant to three figures, as given. In estimating the standardized weights of body components, the weights of moisture and total body were extended to four figures to balance the table (Tables I and II). This has overextended their accuracy. In any respect, while these sources of error may have introduced additional variance, the average results have not been biased.

In the process of removing the stomachs and in preparation for biochemical analysis the accidental introduction of water was carefully avoided. Loss of moisture could not be entirely prevented and must have occurred whenever the samples were exposed to air of less than 100% relative humidity. Trial calculations have shown that in one case, exposure of the sample to the air in a laboratory room heated to 70 F the body lost 2% of its weight in 10 min.

TABLE I. Body composition by weight of subgroup for standard 4-cm fish, 1963 data.

	Geometric means		Adjusted to standard length, 4 cm, $b = 3.0$[a]						
Sample number	Fork length ($\log_{10}$ cm)	Live weight ($\log_{10}$ g)	Total weight (*mg*)	Stomach and contents (*mg*)	Body ex-stomach (*mg*)	Moisture (*mg*)	Protein (*mg*)	Lipid (*mg*)	Unknown (*mg*)
1a	0.5306	$\bar{1}$.3334	353[b]	3.2	349.8	282.3	48.3	9.8	9.4
b	0.5548	$\bar{1}$.4163	361	9.0	352.0	293.9	42.9	8.4	6.8
c	0.5811	$\bar{1}$.5176	381	23.4	357.6	281.1	52.2	11.1	13.2
d	0.6066	$\bar{1}$.5974	383	16.0	367.0	292.9	50.6	12.5	11.0
e	0.5773	$\bar{1}$.4797	358	12.5	345.5	273.3	52.2	10.4	9.6
3a	0.5595	$\bar{1}$.4394	369	12.0	357.0	282.7	53.9	12.1	8.3
b	0.5838	$\bar{1}$.5437	397	14.1	382.9	304.8	55.9	11.1	11.1
c	0.6484	$\bar{1}$.7549	413	16.6	396.4	316.7	57.1	11.9	10.7
d	0.6806	$\bar{1}$.8627	424	13.0	411.0	328.8	60.4	12.3	9.5
e	0.7365	0.0388	432	15.4	416.6	325.4	67.9	15.0	8.3
4a	0.5607	$\bar{1}$.4339	361	10.8	350.2	281.9	54.6	11.6	2.1
b	0.6108	$\bar{1}$.6175	390	13.1	376.9	304.2	52.4	10.6	9.7
c	0.6464	$\bar{1}$.7400	404	12.0	392.0	314.0	59.6	12.5	5.9
d	0.7057	$\bar{1}$.9372	423	13.3	409.7	332.3	59.0	12.7	5.7
e	0.7792	0.1813	447	13.1	433.9	347.6	67.7	16.1	2.5
5a	0.6400	$\bar{1}$.6929	379	4.5	374.5	300.7	57.7	8.2	7.9
b	0.7068	$\bar{1}$.9122	396	9.4	386.6	310.1	60.7	8.5	7.3
c	0.7444	0.0447	414	9.2	404.8	322.2	64.8	11.3	6.5
d	0.8110	0.2596	429	9.7	419.3	322.9	72.1	14.3	10.0
e	0.8437	0.3772	449	10.2	438.8	333.5	77.7	18.4	9.2
7a	0.6653	$\bar{1}$.7790	388	12.3	375.7	300.9	59.4	12.0	3.4
b	0.7428	0.0372	412	11.4	400.6	314.1	70.5	12.0	4.0
c	0.8088	0.2479	424	11.4	412.6	319.4	74.3	14.4	4.5
d	0.8604	0.4396	462	14.5	447.5	347.3	75.6	17.0	7.6
e	0.9058	0.5976	486	13.4	472.6	364.4	80.8	17.0	10.4

(*Continued*)

TABLE I. Body composition by weight of subgroup for standard 4-cm fish, 1963 data.

	Geometric means		Adjusted to standard length, 4 cm, $b = 3.0$[a]						
Sample number	Fork length ($\log_{10}$ cm)	Live weight ($\log_{10}$ g)	Total weight (*mg*)	Stomach and contents (*mg*)	Body ex-stomach (*mg*)	Moisture (*mg*)	Protein (*mg*)	Lipid (*mg*)	Unknown (*mg*)
9a	0.7404	0.0630	445	18.0	427.0	330.0	76.0	14.5	6.5
b	0.7716	0.1569	445	18.9	426.1	331.1	71.2	14.9	8.9
c	0.7952	0.2362	454	18.5	435.5	336.2	71.0	15.7	12.6
d	0.8321	0.3658	474	20.6	453.4	353.2	74.4	18.1	7.7
e	0.8548	0.4334	473	19.1	453.9	348.6	75.3	17.7	12.3
10a	0.7664	0.1511	455	23.8	431.2	338.1	69.9	13.8	9.4
b	0.8044	0.2916	484	21.9	462.1	348.9	79.9	16.2	17.1
c	0.8464	0.4317	500	17.0	483.0	375.3	77.3	20.3	10.1
d	0.8888	0.5739	517	20.9	496.1	385.0	80.9	19.8	10.4
e	0.9395	0.7332	526	22.0	504.0	387.1	82.2	26.2	8.5
11a	0.7688	0.1850	484	23.7	460.3	360.9	75.5	14.7	9.2
b	0.8118	0.3322	505	32.9	472.1	361.6	83.1	17.9	9.5
c	0.8505	0.4504	507	30.2	476.8	368.1	80.1	20.0	8.6
d	0.8820	0.5614	527	37.2	489.8	381.6	77.9	20.1	10.2
e	0.9218	0.6953	544	37.2	506.8	390.7	83.1	22.8	10.2
12a	0.8334	0.4214	534	48.3	485.7	375.9	79.7	20.9	9.2
b	0.8816	0.5725	542	46.8	495.2	381.8	82.7	20.8	9.9
c	0.9086	0.6536	542	40.9	501.1	387.9	84.7	21.5	7.0
d	0.9296	0.7200	546	45.0	501.0	384.8	84.2	35.2	8.5
e	0.9700	0.8338	537	37.5	499.5	381.6	89.4	21.5	7.0
13a	0.7940	0.2774	503	41.3	461.7	367.5	69.3	16.2	8.7
b	0.8363	0.4066	506	37.2	468.8	374.6	75.5	17.8	0.9
c	0.8830	0.5726	536	35.1	500.9	392.2	82.1	19.0	7.6
d	0.9237	0.6828	522	35.2	486.8	378.2	77.4	22.4	8.8
e	0.9525	0.7791	534	32.4	501.6	385.2	88.3	22.6	5.5

(*Continued*)

TABLE I. Body composition by weight of subgroup for standard 4-cm fish, 1963 data.

	Geometric means		Adjusted to standard length, 4 cm, $b = 3.0$[a]						
Sample number	Fork length ($\log_{10}$ cm)	Live weight ($\log_{10}$ g)	Total weight (*mg*)	Stomach and contents (*mg*)	Body ex-stomach (*mg*)	Moisture (*mg*)	Protein (*mg*)	Lipid (*mg*)	Unknown (*mg*)
14a	0.7878	0.2487	491	33.6	457.4	359.5	77.8	16.5	3.6
b	0.8411	0.4285	515	33.0	482.0	375.5	80.0	20.7	5.8
c	0.8771	0.5382	517	32.8	484.2	365.6	84.7	21.3	12.6
d	0.9168	0.6717	534	32.1	501.9	388.5	85.3	23.6	4.5
e	0.9552	0.7839	530	27.9	502.1	384.1	88.9	23.6	5.5
15a	0.7586	0.1350	463	19.4	443.6	343.3	79.4	16.0	4.9
b	0.8212	0.3520	495	22.9	472.1	370.6	78.4	15.1	8.0
c	0.8575	0.4444	476	19.8	456.2	346.7	83.0	18.2	8.3
d	0.9041	0.5976	491	20.9	470.1	357.3	87.9	19.7	5.2
e	0.9494	0.7683	532	25.1	506.9	384.2	90.2	25.3	7.2
16a	0.8067	0.2716	455	17.0	438.0	345.1	71.8	16.6	4.5
b	0.8412	0.3967	478	17.1	460.9	357.7	76.5	18.4	8.3
c	0.8846	0.5414	494	14.7	479.3	365.7	87.7	20.1	5.8
d	0.9039	0.6188	517	16.3	500.7	384.5	88.6	25.0	2.6
e	0.9407	0.7332	521	14.2	506.8	386.7	90.2	24.3	5.6
17a	0.8784	0.5489	524	29.2	494.8	381.0	86.1	21.8	5.9
b	0.9204	0.6964	551	29.4	521.6	395.9	97.5	22.4	5.8
c	0.9399	0.7444	538	28.7	509.3	379.9	98.3	23.4	7.7
d	0.9526	0.7963	555	30.5	524.5	393.4	98.1	29.4	3.6
e	0.9792	0.8708	548	29.7	518.3	390.3	95.4	29.5	3.1
18a	0.9409	0.7611	555	43.6	511.4	398.4	91.5	14.8	6.7
b	0.9749	0.8521	542	44.2	497.8	384.3	85.6	12.9	15.0
c	0.9891	0.8976	545	44.5	500.5	387.9	100.0	11.5	1.1
d	0.9992	0.9246	541	46.6	494.4	387.1	91.0	11.9	4.4
e	1.0208	1.0026	558	46.9	511.1	395.1	96.6	12.8	6.6

(*Continued*)

TABLE I. Body composition by weight of subgroup for standard 4-cm fish, 1963 data.

(*Concluded*)

Sample number	Geometric means: Fork length ($\log_{10}$ cm)	Geometric means: Live weight ($\log_{10}$ g)	Adjusted to standard length, 4 cm, $b = 3.0$[a]: Total weight (*mg*)	Stomach and contents (*mg*)	Body ex-stomach (*mg*)	Moisture (*mg*)	Protein (*mg*)	Lipid (*mg*)	Unknown (*mg*)
19a	0.9160	0.6156	472	12.8	459.2	351.7	91.8	13.3	2.4
b	0.9512	0.7338	486	12.7	473.3	367.3	87.1	12.3	6.6
c	0.9711	0.7954	488	13.3	474.7	372.2	87.8	10.4	4.3
d	0.9945	0.8658	488	12.5	475.5	369.5	86.5	12.4	7.1
e	1.0226	0.9487	487	12.9	474.1	367.0	92.4	13.3	1.4
20a	0.9184	0.6294	479	16.4	462.6	360.4	84.2	11.1	6.9
b	0.9490	0.7300	489	15.0	474.0	363.1	90.5	12.8	7.6
c	0.9634	0.7788	495	16.7	478.3	373.1	83.2	12.9	9.1
d	0.9792	0.8266	496	16.7	479.3	368.6	87.7	13.4	9.6
e	1.0086	0.9307	514	18.1	495.9	383.8	90.7	13.4	8.0
21a	0.9070	0.6028	488	24.8	463.2	361.8	81.5	13.9	6.0
b	0.9309	0.6850	499	29.6	469.4	357.2	90.1	14.6	7.5
c	0.9579	0.7774	512	28.4	483.6	372.4	89.9	14.5	6.8
d	0.9746	0.8188	503	27.1	475.9	367.4	89.5	12.4	6.6
e	0.9956	0.8903	512	28.3	483.7	374.4	94.8	13.5	1.0

[a] $(\log 4 - \log l)\ 3 + \log w = \log \text{standard } \hat{w}$.
[b] $(.6021 - .5306)\ 3 + \bar{1}.3334 = \bar{1}.5482$; antilog $\bar{1}.5482 = 0.353$ g or 353 mg.

A loss of 2% of the body weight in the form of moisture results in approximately a 2% overestimation of protein and lipid but in only 0.5% underestimation of moisture. Similarly, a 10% lost of body weight results in the overestimation of protein and lipid by 11% and the underestimation of moisture by but 2.4%. The magnitude of this source of error, while believed to be generally less than 5%, is unknown and biases the resulting averages.

Theoretically, small fish, because of their greater relative surface area, would lose water at a greater relative rate than large fish. The conditions of exposure to air were not standard. Large fish may or may not have been exposed to the air for longer periods than the small fish, and the relative humidity of

TABLE II. Body composition per sample average adjusted to standard 4-cm fish, 1964 data.

	Geometric means		Adjusted to standard length, 4 cm, $b = 3.0$					
Sample number	Fork length ($\log_{10}$ cm)	Live weight ($\log_{10}$ g)	Total weight (*mg*)	Stomach and contents[a] (*mg*)	Moisture (*mg*)	Protein (*mg*)	Lipid (*mg*)	Unknown (*mg*)
1	0.5843	$\bar{1}$.4624	328	...	276.8	36.1	10.8	4.3
10	0.6550	$\bar{1}$.6926	350	11.8	287.4	46.2	14.4	2.0
22	0.6800	$\bar{1}$.7798	352	31.4	290.0	46.5	15.1	0.4
32	0.7195	$\bar{1}$.9065	358	29.8	290.0	51.6	16.8	−0.4
42	0.7491	$\bar{1}$.9913	355	26.9	290.0	50.4	14.9	−0.3
60	0.8335	0.3075	411	26.3	330.0	58.8	13.2	9.0
68	0.9499	0.6825	436	41.6	343.6	72.4	11.3	8.7
70	0.8973	0.5431	455	28.9	366.3	66.0	8.2	14.5
124	0.9538	0.6966	438	13.2	346.9	74.5	5.7	10.9
125	0.9053	0.5405	385	15.7	309.5	60.8	5.8	8.9

[a]This figure consistently too high. Total weights were considered as ex-stomach and contents; in reality they include the empty stomach. The error is between 0.1 and 0.2 mg, according to the fullness of the stomachs and has negligible effect on subsequent calculations.

the laboratory was not controlled. In preparation for biochemical analysis the fish were finely ground and no distinction between rates of evaporation can be made in regard to large and small fish. The 90 separate treatments of the samples of 1963 would tend to obscure any size-specific error due to water loss.

In 1964, stomachs were not dissected thus eliminating one of the opportunities for water loss. On the other hand, the samples were not subdivided and only 10 determinations were made representing the wild population. In these data the variable loss of water has probably altered both the positions and slopes of the apparent regressions for there is no opportunity for duplication of size-groups. The 1964 material is considered to be useful only for a comparison of gross features between the years.

In correcting the lipid determinations of 1964 a coefficient of 2.6 was applied. In some cases (Appendix C, Table II) this has led to minus quantities in the unknown fraction of body weight. The correction used is in some cases, therefore, a little too large.

In the presentations that follow then, it is expected that the variance apparent around a general regression of weight on length reflects minor random error of measurement as well as true biological variability. Biological variability

is not adequately represented because mean values have been used. Values for individual fish would undoubtedly show a wider dispersion.

RESULTS

The relationships between body weight, moisture, protein, and lipid, estimated in Tables I and II, are graphically presented in Fig. 2. Logarithmic axes are used, hence where the relationships are allometric the plots will be linear. Where the relationships are isometric the slope coefficients will be zero. Relative variance is directly comparable among the plots of the relationships. The 1963 data are represented by the open circles, the 1964 date by the closed squares.

Several features are readily recognized. The length–body weight relationship is not initially isometric. From the smallest size to a length of 7–8 cm weight did not increase proportionately to the increase in cube of length, i.e. the allometric relationship is positive; the fish were growing relatively more obese with increase in size. At lengths greater than 7–8 cm the relationship is approximately isometric, i.e. the fish grew without change in relative weight.

None of the plots of length–body–constituent parallel that of the length–weight relationship. Evidently moisture, protein, and lipid were maintained in no constant relationship with each other. To facilitate the comparison of these relationships, slope coefficients have been calculated from and are shown (Fig. 2) connecting mean values of two groups: (1) 12 sets of values for subgroups of mean lengths less than 4.5 cm, and (2) 12 sets of values for subgroups of mean lengths between 6.5 and 7.5 cm.[5] The calculations of these reference lines are given in Table III.

The length–moisture relationship resembles that for length–body weight in initially displaying positive allometry and changing at 7–8 cm to an approximately isometric relationship. The two slope coefficients are noticeably different, however, moisture, prior to 7–8 cm length, becomes progressively a smaller fraction (from 80.2 to 77.6%) of the total body weight as evidenced by the lesser slope. The length–protein relationship differs from the above in three essential features: (1) the relationship shows positive allometry throughout the size range although there is an inflection; (2) the inflection in slope appears to have occurred at a smaller size (at about 5.5–6.0 cm); (3) the slope of the reference line (Table III) is higher than that for weight. Protein, with increase of size, progressively became a higher proportion of total body weight. In the averages of the two groups used to calculate the reference lines, protein increased from 14.5 to 16.7% of body weight. Thus, the total body weight, increasing with positive allometry, was largely the sum of water and protein, the latter increasing at a faster relative rate than the former.

[5]Since both length and weight are subject to error the standard least-squares solution for regression statistics is inappropriate. We have used this simple technique to estimate these reference lines (see Wald, 1940).

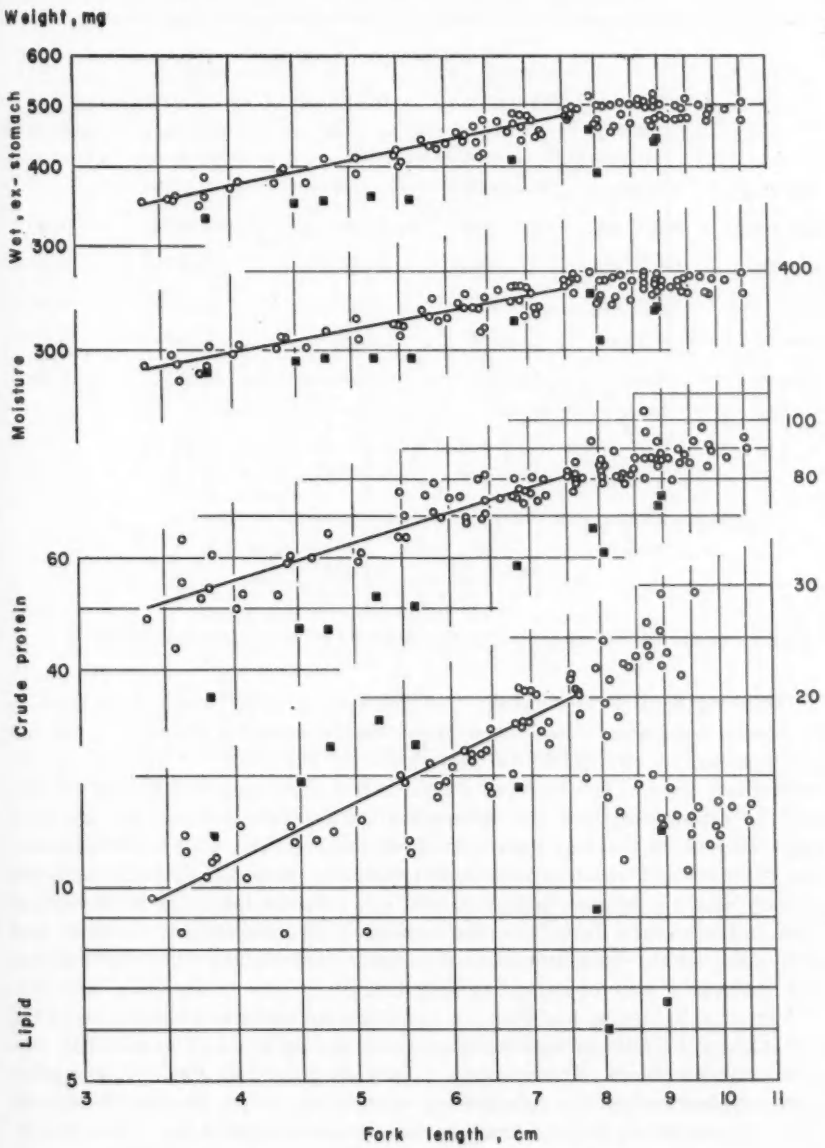

FIG. 2. Relationship among length and standardized weights of body ex-stomach, moisture, protein and lipid (both axes $\log_{10}$).

TABLE III. Estimating the slopes of those 1963 data from fish less than 8.0 cm plotted in Fig. 2.

	Averages (n = 12) 3.4–4.5-cm group		Averages (n = 12) 6.5–7.5-cm group		Regression coefficients[a]
	Size	($\log_{10}$)	Size	($\log_{10}$)	
Fork length	3.90 cm	0.5910	6.98 cm	0.8440	...
Body weight	366.8 mg	2.5644	464.9 mg	2.6674	0.407
Moisture	294.0 mg	2.4683	360.6 mg	2.5570	0.350
Protein	53.12 mg	1.7253	77.79 mg	1.8909	0.654
Lipid	10.85 mg	1.0354	18.55 mg	1.2683	0.920
Unknown	8.81 mg	0.9450	8.00 mg	0.9031	−0.166

[a]Regression coefficient $b = \dfrac{\log\left[\frac{1}{n}\sum_{l_1=3.4}^{l_n=4.5} \chi_l\right] - \log\left[\frac{1}{n}\sum_{l_1=6.5}^{l_n=7.5} \chi_l\right]}{\log\left[\frac{1}{n}\sum_{l_1=3.4}^{l_n=4.5} l_l\right] - \log\left[\frac{1}{n}\sum_{l_1=6.5}^{l_n=7.5} l_l\right]}$

where χ = the standardized weight (Table I) of the body or of one of its constituents.

The length–lipid relationship does not appear to be linear. As a working hypothesis, we assume that this relationship is basically allometric, but has been modified by environmental factors during the course of growth. At the intermediate sizes of 4–6 cm lipid weight is less than expected as may be seen from the departures from the reference line. Between 7.5 and 9.5 cm lipid values fell into two clearly separated levels, high or low. This separation into high or low levels appears to be related to calendar time, the division occurring at about May 27. At sizes larger than 9.5 cm only the lower lipid values were found. In contrast to the length relationships with body weight, moisture, and protein, the length–lipid relationship is then marked by severe fluctuations. These fluctuations are of immediate interest.

Variation in food availability is a logical choice for an environmental factor which affects the relative lipid component of the body. Food availability was not directly measured. The amount of food contained in the fish stomachs, however, reflects what was recently eaten and may reflect relative differences in food availability. These estimates are given in Appendices A and B. In Fig. 3 (bottom half) extreme subsample values for each day of 1963 observations are shown as functions of calendar time. The relatively wide range in values for fish taken on April 21 undoubtedly reflects the unfed condition of entering fry mixed with fry already present and having fed. Similar wide ranges

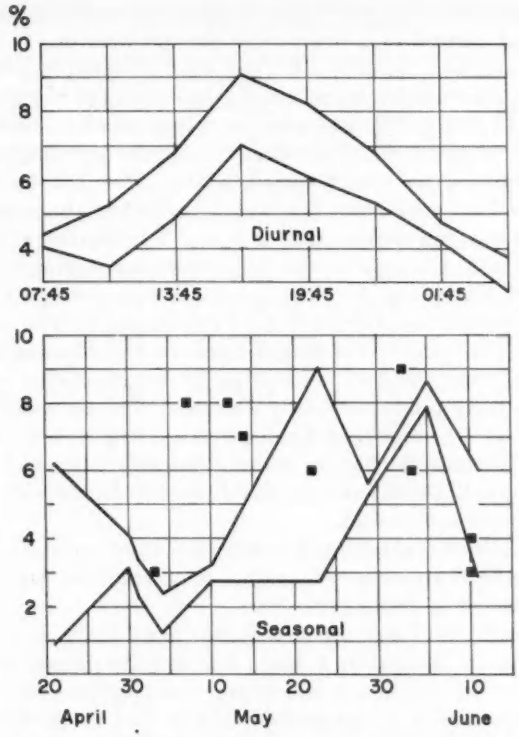

FIG. 3. Diurnal (upper) and seasonal (lower) changes in relative stomach weights of juvenile pink salmon.

in values are indicated for samples obtained on May 23 and on June 11. These ranges can be shown to reflect diurnal variation in feeding. Data for May 23–24 samples, obtained at 3-hr intervals, are presented in Fig. 3 (upper half). A diurnal feeding cycle is evident with peak feeding occurring in the afternoon and the low in early morning. Samples taken on June 11 confirm the pattern but this diurnal series is not complete. The observations of 1964 display initial and final low values with mid-seasonal high values of food ingestion. Thus, in general, the two seasons were similar.

The diurnal cycle of food intake may be related to changes in the relative weight of the body and its constituents. A positive but nonsignificant correlation ($r = 0.46$; df = 6) exists, however, between the paired average sample values for length and relative stomach content (Appendix A, samples 9–16). Since the length–weight relationships for total body weight and the weight of each constituent show positive allometry, a positive correlation also exists between the standardized weights of the components and the relative stomach content. Thus, the effects of size must be removed from the data before a

meaningful comparison can be made. Sample average lengths are presented in Table IV and plotted as a function of diurnal time in Fig. 4 (upper). All data from the samples of May 23–24, 1963, were adjusted to a standard length of 7.5 cm. For body weight (ex-stomach) the estimated regression coefficient value of $b = 3.41$ (Table III) was used for subsamples having average lengths of less than 7.5 cm and $b = 3.0$ for those having average lengths greater than 7.5 cm. For adjusting the length–moisture data, $b = 3.35$ for $l < 7.5$ cm and $b = 3.0$ for $l > 7.5$ cm were used. The length–protein relationship was assumed to be linear for lengths greater than 5.5 cm. The regression coefficient was estimated from the averages of the 16 subsamples having average lengths between 5.5 and 6.5 cm and the 19 subgroups having average lengths between 8.5 and 9.5 cm. Details are given in Table IV (footnote c). The resulting regression coefficient is $b = 3.49$. The length–lipid relationship was assumed to be linear, however, data obtained after May 29 were excluded because of the obvious shift in body lipids previously discussed. The regression coefficient is estimated from the 19 subsamples having average lengths between 5.5 and 6.5 cm and the 10 subsamples having average lengths between 8.5 and 9.5 cm taken prior to June 1. Details are given in Table IV (footnote d). The resulting regression coefficient is $b = 4.25$.

With these estimates of regression coefficients the corresponding constituent values were modified to estimate weights at a standard length of 7.5 cm. Results are presented in Table IV.

Data presented in Table IV are shown graphically in Fig. 4 (lower). Average values are connected by a heavy line and the ranges are indicated by the height of the column above and below the averages. A rise and fall of relative body moisture is a pronounced feature and is obviously correlated with the diurnal feeding cycle shown in Fig. 3. As a consequence, the body weight also shows a pronounced diurnal change while relative protein and relative lipid changes are so small that they can have but little effect on body weight *per se*. Relative variation in protein and lipid is much greater than that of body weight or moisture, however, these features are subject to relatively large errors of measurement.

The relative weight of protein, which apparently was little affected by diurnal sampling and was allometric with regard to length independent of food ingested or calendar time, will serve as a reference base to compare lipid levels with food ingested. Lipid–protein ratios are compared with stomach weight–protein ratios in Table V. The ratios are plotted in Fig. 5.

In Fig. 5 the 1963 data are plotted as circles, the 1964 data as squares. Solid symbols indicate data acquired on the same day, i.e. the series of seven solid circles (the first, representing sample 9, also represents sample 15) represents the diurnal series of observations obtained on May 23–24. These data *per se* indicate a situation where a relatively large variation in stomach content has not affected the lipid level, i.e. the series forms a narrow band which parallels the abscissa. Thus, variation in diurnal time of sampling tends to obscure the relationship between the two ratios. Nevertheless, the 1963

TABLE IV. Estimated weights of the body and its constituents at a standard length of 7.5 cm, May 23–24, 1963, diurnal series.

Time	Sample number	Fork length (*cm*)	Body[a] ex-stomach (g)	Moisture[b] (g)	Protein[c] (g)	Lipid[d] (g)
0745	9a	5.50	3.20	2.42	0.583	0.141
	b	5.92	3.10	2.37	0.527	0.133
	c	6.24	3.10	2.36	0.512	0.130
	d	6.80	3.11	2.41	0.515	0.135
	e	7.16	3.50	2.34	0.509	0.124
	Average:	6.32	3.11	2.38	0.529	0.133
1045	10a	5.85	3.15	2.43	0.521	0.124
	b	6.38	3.26	2.43	0.570	0.131
	c	7.02	3.27	2.53	0.526	0.145
	d	7.75	3.23	2.54	0.525	0.126
	e	8.72	3.13	2.55	0.504	0.144
	Average:	7.14	3.21	2.50	0.529	0.134
1345	11a	5.88	3.36	2.59	0.561	0.132
	b	6.48	3.31	2.51	0.585	0.142
	c	7.10	3.22	2.48	0.543	0.142
	d	7.62	3.21	2.51	0.509	0.130
	e	8.38	3.20	2.58	0.521	0.132
	Average:	7.09	3.26	2.53	0.544	0.136
1645	12a	6.82	3.33	2.56	0.550	0.155
	b	7.62	3.24	2.51	0.541	0.135
	c	8.11	3.20	2.56	0.538	0.129
	d	8.52	3.14	2.54	0.522	0.133
	e	9.34	3.01	2.51	0.526	0.108
	Average:	8.08	3.18	2.54	0.535	0.132
1945	13a	6.24	3.29	2.59	0.500	0.135
	b	6.86	3.20	2.55	0.520	0.131
	c	7.64	3.28	2.59	0.538	0.123
	d	8.40	3.07	2.50	0.483	0.128
	e	8.98	3.08	2.54	0.534	0.119
	Average:	7.62	3.18	2.55	0.515	0.127

(*Continued*)

TABLE IV. Estimated weights of the body and its constituents at a standard length of 7.5 cm, May 23–24, 1963, diurnal series. — (*Concluded*).

Time	Sample number	Fork length (*cm*)	Body[a] ex-stomach (g)	Moisture[b] (g)	Protein[c] (g)	Lipid[d] (g)
2245	14a	6.16	3.28	2.55	0.566	0.140
	b	6.94	3.28	2.54	0.548	0.151
	c	7.54	3.18	2.41	0.557	0.140
	d	8.26	3.18	2.56	0.537	0.138
	e	9.04	3.07	2.53	0.536	0.124
	Average:	7.59	3.20	2.52	0.549	0.137
0145	15a	5.77	3.26	2.51	0.597	0.147
	b	6.64	3.28	2.55	0.549	0.128
	c	7.22	3.06	2.32	0.559	0.127
	d	8.04	3.02	2.36	0.560	0.120
	e	8.94	3.12	2.54	0.548	0.135
	Average:	7.32	3.15	2.46	0.563	0.131
0445	16a	6.40	3.08	2.40	0.511	0.134
	b	6.94	3.14	2.42	0.524	0.134
	c	7.68	3.13	2.41	0.572	0.129
	d	8.02	3.21	2.54	0.566	0.152
	e	8.74	3.14	2.55	0.553	0.133
	Average:	7.56	3.14	2.46	0.545	0.136

[a] b_{tw} = 3.41 (Table III value + 3.0).
[b] b_{tm} = 3.35 (Table III value + 3.0).
[c] Where χ_l = average weight of protein for subsample at a standard length of 4 cm (Table I) the regression coefficient (b) is estimated as

$$b = \frac{\log\left[\frac{1}{n}\sum_{l_1=8.5}^{l_n=9.5}\chi_l\right] - \log\left[\frac{1}{n}\sum_{l_1=5.5}^{l_n=6.5}\chi_l\right]}{\log\left[\frac{1}{n}\sum_{l_1=8.5}^{l_n=9.5}l_l\right] - \log\left[\frac{1}{n}\sum_{l_1=5.5}^{l_n=6.5}l_l\right]} + 3.0 = 3.49.$$

[d] Same as in footnote C where χ = average weight of lipid but excluding samples obtained in June. b = 4.25.

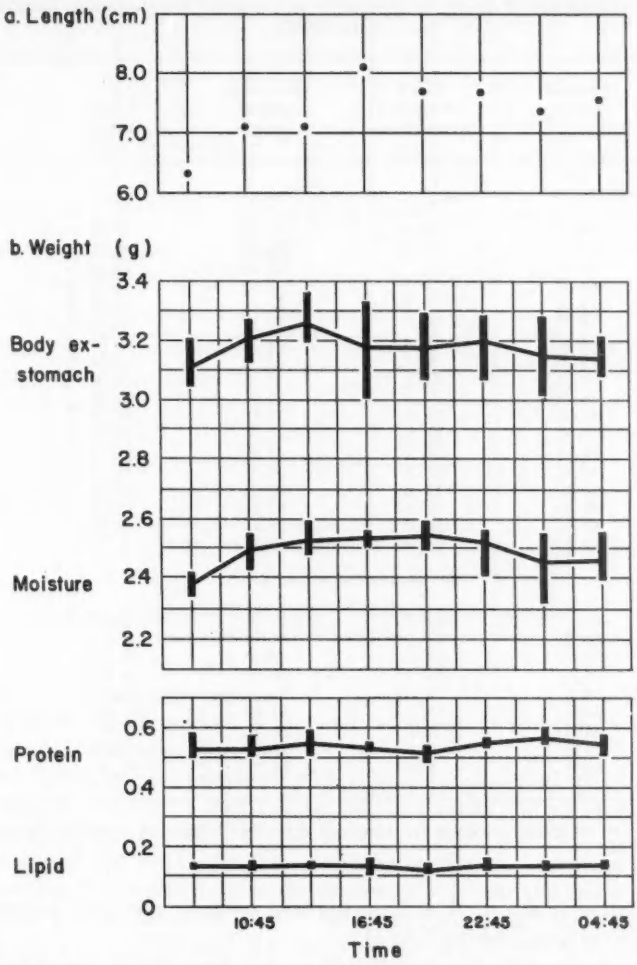

FIG. 4. Diurnal changes in body weight and the weights of body constituents.

data form two clearly independent fields separated in time. The samples taken in May form the upper and (connected by a dashed line) the samples obtained in June form the lower. In the upper correlation field a positive slope is apparent indicating that, in general, the more food ingested, the higher the relative amount of body lipid. After the shift in body lipids the observations suggest a resumption of positive correlation but in a distinct and lower plane. The 1964 data appear to extend the upper correlation field of the 1963 data into regions of

TABLE V. Calculation of ratios of lipid–protein and stomach weight–protein for 1963 and 1964 samples[a].

Sample number	Protein weight (*mg*)	Lipid weight (*mg*)	Stomach weight (*mg*)	Lipid protein	Stomach protein
1963					
1	49.2	10.4	12.8	0.21	0.26
3	59.0	12.5	14.2	0.21	0.24
4	59.0	12.7	12.5	0.22	0.21
5	66.6	12.1	8.6	0.18	0.13
7	72.1	14.5	12.6	0.20	0.18
9	73.6	16.2	19.0	0.22	0.26
10	78.0	19.3	21.1	0.25	0.27
11	79.9	19.1	32.2	0.24	0.40
12	84.1	21.6	43.7	0.26	0.52
13	78.5	19.6	36.2	0.25	0.46
14	83.3	21.1	31.9	0.25	0.38
15	83.8	18.9	21.6	0.22	0.26
16	83.0	20.9	15.9	0.25	0.19
17	95.1	25.3	29.5	0.27	0.31
18	92.9	12.8	45.2	0.14	0.49
19	89.1	12.3	12.8	0.14	0.14
20	87.3	12.7	16.6	0.15	0.19
21	89.2	13.8	27.6	0.15	0.31
1964					
10	46.2	14.4	11.8	0.31	0.26
22	46.5	15.1	31.4	0.32	0.68
32	51.6	16.8	29.8	0.33	0.58
42	50.4	14.9	26.9	0.30	0.53
60	58.8	13.2	26.3	0.22	0.45
68	72.4	11.3	41.6	0.16	0.57
70	66.0	8.2	28.9	0.12	0.44
124	74.5	5.7	13.2	0.08	0.18
125	60.8	5.8	15.7	0.09	0.26

[a]Based on estimated weights for standard 4-cm fish assuming isometry as presented in Tables I and II.

greater relative levels of food ingested and confirm the shift of body lipid, which again occurred during late May.

These comparisons are interpreted as follows. During the early stage of sea life the quantity of food ingested had no apparent effect on the length–protein relationship but the relative amounts of body lipid were directly affected. A shift to lower lipid levels occurred about the end of May which was independent of food ingested. Following the shift relative lipid levels may have resumed dependence upon the amount of food ingested.

To this point the data studied have represented the wild populations. Relative lipid levels were apparently influenced by the amount of food ingested but no opportunity to observe the effects of a severely restricted food supply was found in the natural conditions. From data obtained on fish held under various conditions of food limitation it is possible to infer the effects of restrictive

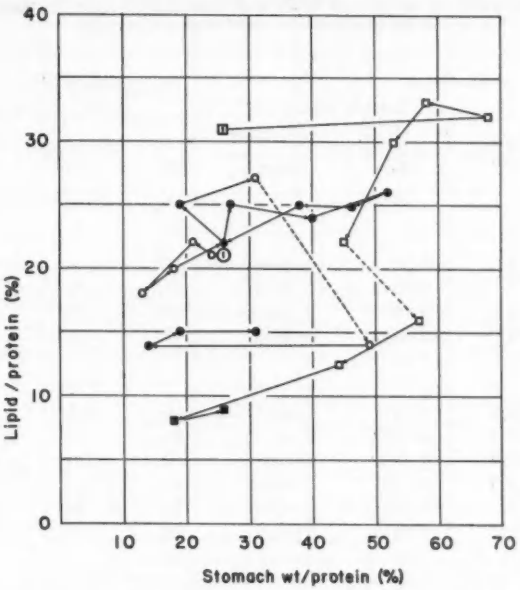

FIG. 5. Lipid–protein ratios compared with stomach weight–protein ratios.

food supply on total body weight and the weight of the body components. Basic data have been presented in Appendices C and D and the conditions of the experiments have been described.

In both years, the experimental groups were composed of fry of relatively uniform size (Appendices C and D). In 1963 the range of fork lengths of the experimental population was from 3.1 to 4.4 cm and in 1964, 3.5 to 4.5 cm. Growth in length did not occur during either experiment. In both cases, these populations were obtained from single catches of fry near the estuary and the range in size is considered as mainly reflecting biological variation within a single age rather than the effects of multiple sea ages included in the sample. Accordingly, it is appropriate to assume isometry for purposes of standardizing weights at a common length rather than use any of the allometric relationships previously presented. The results of this transformation are presented in Table VI. The 1964 data result from single-sample determinations. The 1963 data result from the averaging of five subsamples for each sample, hence are more reliable than those of 1964. The results are presented in Fig. 6, plotted on logarithmic axes.

The series of 1963 (open circles) shows the effects of rather severe crowding and of limiting the food supply. Under conditions of approximately 10,000/m^3 for 7 days, body weight decreased about 12% or 43 mg standard measure. This decrease was due to a loss of 15% of body moisture (42 mg), a loss of 22%

TABLE VI. Comparison of weights of body and constituents after various times of holding under conditions of restricted food supply.

Sample number	Days held	Sample average		Standardized to 4.0 cm fork length			
		$\log_{10}$	$\log_{10}$	Body (*mg*)	Moisture (*mg*)	Protein (*mg*)	Lipid (*mg*)
1963							
1	0	0.5701	$\bar{1}$.4689	355	285	49.3	10.4
2	7	0.5695	$\bar{1}$.3997	312	243	52.2	8.1
6	16	0.5873	$\bar{1}$.5001	346	282	50.7	6.7
8	20	0.5895	$\bar{1}$.4984	330	267	48.4	7.7
1964							
1	0.2	0.5831	$\bar{1}$.4695	336	284	37.0	12.1
2	1.2	0.5863	$\bar{1}$.4631	324	274	34.7	11.0
3	2.2	0.5834	$\bar{1}$.4421	315	264	40.0	11.0
4	3.5	0.5907	$\bar{1}$.4356	295	254	30.4	7.7
5	8.2	0.5842	$\bar{1}$.3770	270	240	22.1	4.6
6	18.2	0.5813	$\bar{1}$.3186	241	210	22.8	3.6

of lipid (2.3 mg), and a gain of 6% of protein (2.9 mg). At the end of 7 days the population was thinned to a density of about 200/m^3. After 9 days under these conditions body weight had increased by about 11% (34 mg standard measure). This increase was due to a 16% increase in moisture (39 mg), a 3% decrease in protein (1.5 mg) and a 17% decrease in lipid (1.4 mg). A further decrease in density to approximately 100/m^3 for a period of 4 days produced a 5% decrease in body weight (16 mg), resulting from a 5% decrease in moisture (15 mg), a 5% decrease in protein (2.3 mg), and a 15% increase in lipid (1.0 mg).

Clearly the significant changes under the stated conditions involved gain or loss of relatively large amounts of moisture. Relative to its own magnitude the changes in water, while great, were surpassed by changes in lipid. The changes in protein are relatively minor and may be due largely to measurement error. Changes in moisture and lipid are considered significant. It is noted that under the conditions of 200/m^3 body weight returned nearly to its original value even though the decline in lipid continued. Thus, under the most severe conditions, moisture and lipid were lost. Under less severe conditions moisture was gained, lipid was lost. Under even less severe conditions moisture was lost and lipid was gained. Two relationships bearing on the apparent condition as judged by relative body weight are noted: (1) weight increased while lipid decreased, and (2) weight decreased while lipid increased.

The 1964 experimental population was subjected to total conditions of starvation except for cannibalism which developed late in the experiment. Loss of water and hence loss of body weight roughly parallels that of the initial

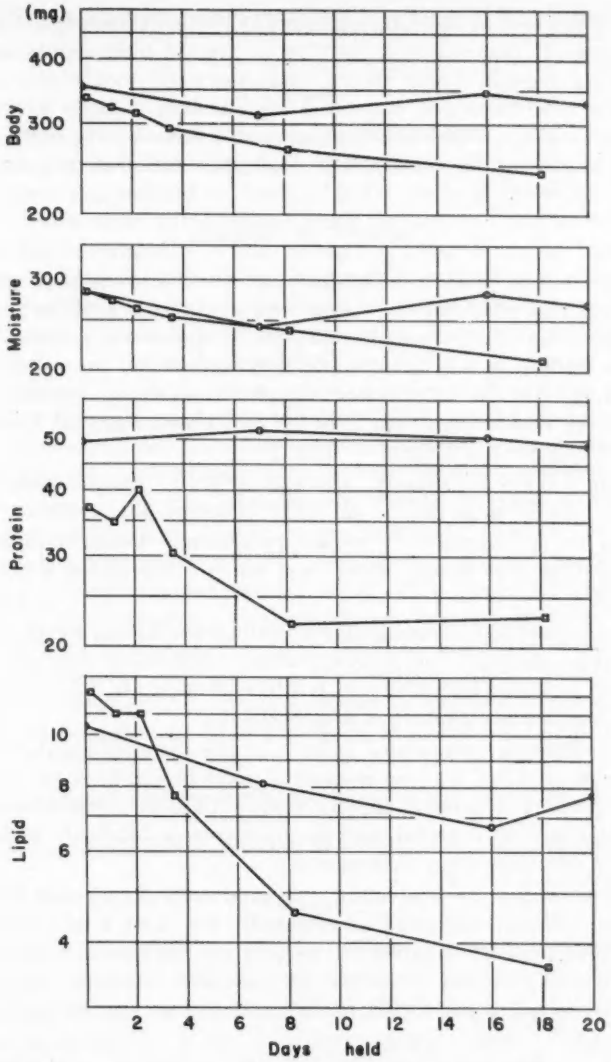

FIG. 6. The effects of holding pink salmon under conditions of restricted food supply on body and body constituent weights.

conditions of 1963, but in contrast is accompanied by a loss of both protein and lipid. The general decline continued throughout the experiment but at a decreasing relative rate of loss. An exception is the leveling or slight increase of protein during the final 10 days. This was during the period of death and cannibalism, and protein was therefore available for ingestion.

These observations indicate, without any precise knowledge of the amount of food ingested, that the first reaction to limited food supply is a general suppression of growth. Under severe conditions body weight may be lost but intermediate conditions may involve a maintenance of body weight through an increase of water. Conditions leading to the loss of body protein were not recognized in the natural environment during the period of this study.

RESUME OF RESULTS

Changes taking place in body composition of juvenile pink salmon have been studied from the time they enter the sea in mid April as fry until the time they leave the inside waters in mid June. While food availability fluctuated during this period, it was always sufficient apparently for normal relative-growth processes in the natural environment. Under these conditions the fish grew relatively heavier to a length of 7.5 cm. Further growth in weight was isometric with growth in length.

The main body constituent was water. Water progressively became a lesser proportion of total weight until 7.5 cm length was achieved, thereafter it remained an approximately constant proportion. A diurnal fluctuation of moisture content was noted, which was directly correlated with a diurnal feeding cycle.

Protein formed an increasingly larger proportion of body weight throughout the size range studied. The length–protein relationship was not constant, however, but an initial high degree of positive allometry to 5.5 cm was followed by a lesser but positive allometric relationship.

Relative lipids are postulated to be basically correlated with relative protein but were modified by the amount of food ingested. A shift from high to low lipid content occurred at the end of May. This shift was not accompanied by a decrease of food ingested, and occurred over a relatively wide range in size during a relatively short time-period.

The first reaction to a severely restricted food supply was loss of lipid and moisture. Protein remained at essentially the same level. At less severe conditions (but preventing growth in length) fish continued to lose lipid but, body weight was returned to normal by increased moisture content. Under conditions of complete starvation protein was lost as well as lipid and body moisture.

There were, then, two size-specific inflections which occurred in the relative growth lines of the juvenile pink salmon, one at a length of 5.5 cm (protein-length) and the second at a length of 7.5 cm (moisture-length). A third change involved a decrease in body lipids which occurred during a discrete calendar time-period and was not a function of size *per se* but coincided with the size range 8.0–9.5 cm.

These inflections and shifts all affected the length–weight relationship, however, body moisture had the most significant effect. Thus, body weight

was ostensibly positively allometric with length to a size of 7.5 cm and isometric thereafter.

DISCUSSION

A direct comparison with Kizevetter's (1948) data can only be made by averaging some of the percentage body composition data given in Appendix A. A comparison is presented as Table VII. To typify pink salmon fry at the start of marine life those subsamples having average fork lengths of less than 4.0 cm have been averaged. For the premigration stage, samples taken for the diurnal series on May 23–24 have been averaged. For the migratory stage those samples obtained in June were averaged. Two points of difference are noted: (a) the progressive decrease in relative moisture is more pronounced in the pink salmon than in the sockeye salmon, and (b) the percentage of protein generally increased in pink salmon, while in sockeye salmon the percentage of protein first decreased and then increased. The increase in body lipid followed by a decrease at the migratory stage is a similarity among these species and presumably also with Atlantic salmon. Thus, we infer that this migratory stage in pink salmon may be equivalent to the smolt stage of Atlantic and sockeye salmon although no adaptation to changes in salinity is involved.

These changes in relative body composition obviously occurred in an orderly and progressive manner with growth, and it appears evident that the relative decrease in moisture accompanied by relative increases in protein and lipid, in combination, can be considered as a general feature of their ontogenesis. By this we imply, in respect to gross moisture, protein, and lipid composition, a size-specific progressive change in chemical composition within the concept of homeostasis. Fluctuations in environmental opportunities for

TABLE VII. Comparison between chemical composition of pink salmon and sockeye salmon[a] at various life-history events.

	Average weight (g)	Per cent of body weight			
		Moisture	Protein	Lipid	Total
Sockeye fry (3 months)	1.32	80.23	16.10	1.97	98.30
Pink fry (start of sea life)	0.29	80.2	14.4	3.0	97.6
Fingerling sockeye (presmolt)	10.0	79.32	13.35	6.02	98.69
Fingerling pinks (premigration)	3.41	77.3	16.9	4.1	98.3
Smolt sockeye (seaward migration)	21.2	79.06	15.21	3.67	97.94
Migrant pinks (oceanward migration)	6.61	77.4	18.6	2.7	98.70

[a]Based on Table V from Kizevetter, 1948. Values were derived from round fish (including stomach and contents).

growth are imposed upon this basic plan, but in the natural environment these found expression only in the lipid deposits.

A net loss of protein was observed only under conditions of imposed starvation, i.e. these conditions did not provide even the opportunity for the maintenance of status quo. This observation is substantiated by the experimental results of Phillips et al. (1959). These authors also reported rhythmic changes in water content, and hence changes in the percentage protein content, regardless of experimental conditions imposed. Such rhythms undoubtedly account for much of the variance evident in our presentations.

Total lipid content was confined between 2 and 6% of the total body weight within the sizes of pink salmon studied. Part of this range is attributed to ontogenetic increases in relative lipid. Some of the fluctuations in percentage lipid content are attributable to diurnal variation in total body weight resulting from fluctuations in body moisture. Under even more severe conditions, experimentally imposed, the catabolic consumption of lipid was accompanied by an uptake in water.

Thus, condition factors, whether based on isometry or allometric slope values calculated from sample statistics, derived from length–weight relationships, would appear to be quite insensitive indicators of the fish's well-being and extremely difficult to interpret into terms of environmental opportunities for growth.

Indeed, Martin (1949) has shown the large degree to which body form of fishes is determined by the size attained at the end of a growth stanza marked by an inflection in the ontogenetically fixed slopes of relative growth lines. Within a growth stanza body form was largely independent of environmental factors other than severe conditions of starvation.

Within the confines of the present study, it is suggested that relative stomach content, noting diurnal feeding rhythms, provides a more sensitive and easily interpreted measure. Problems of food quality, parasitism, and disease, however, undoubtedly complicate the interpretation of this simple statistic.

Points of inflection have been noted in the relative growth lines for moisture and protein. Because of the nature of the data, i.e. the inclusion of several sizes and/or sea-ages in each subsample, considerable variance is contained within the sample statistics. A more precise definition of the allometric lines and points of inflection for body constituents re size and/or sea-age must await the opportunity for closer control of the independent parameter.

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(Appendices A to C *follow*)

APPENDIX A. Sample statistics and percentage body composition of juvenile pink salmon, central British Columbia, 1963.

Date of sample 1963	Sample number	Mean fork length (*cm*)	Range of lengths (*cm*)	Mean live weight (g)	Stomach and contents (%)	Based on ex-stomach live weight			
						Moisture (%)	Protein (%)	Lipid (%)	Unknown (%)
April 21	1a	3.40	3.2– 3.5	0.218	0.92	80.7	13.8	2.8	2.7
	b	3.59	3.5– 3.7	0.262	2.48	83.5	12.2	2.4	1.9
	c	3.82	3.6– 4.1	0.335	6.15	78.6	14.6	3.1	3.7
	d	4.04	3.6– 4.3	0.390	4.18	79.8	13.8	3.4	3.0
	e	3.78	3.5– 4.4	0.309	3.48	79.1	15.1	3.0	2.8
April 30	3a	3.63	3.4– 3.9	0.277	3.25	79.2	15.1	3.4	2.3
	b	3.84	3.6– 4.1	0.351	3.56	79.6	14.6	2.9	2.9
	c	4.46	4.3– 4.7	0.572	4.02	79.9	14.4	3.0	2.7
	d	4.80	4.6– 5.0	0.734	3.07	80.0	14.7	3.0	2.3
	e	5.47	4.9– 6.4	1.152	3.56	78.1	16.3	3.6	2.0
May 1	4a	3.64	3.3– 3.9	0.278	2.34	80.5	15.6	3.3	0.6
	b	4.08	3.9– 4.3	0.417	3.36	80.7	13.9	2.8	2.6
	c	4.44	4.2– 4.7	0.554	2.98	80.1	15.2	3.2	1.5
	d	5.08	4.8– 5.3	0.872	3.15	81.1	14.4	3.1	1.4
	e	6.04	5.7– 8.5	1.615	2.94	80.1	15.6	3.7	0.6
May 4	5a	4.38	3.7– 4.8	0.504	1.19	80.3	15.4	2.2	2.1
	b	5.10	4.8– 5.5	0.822	2.37	80.2	15.7	2.2	1.9
	c	5.56	5.1– 6.1	1.122	2.23	79.6	16.0	2.8	1.6
	d	6.48	6.0– 6.8	1.828	2.27	77.0	17.2	3.4	2.4
	e	6.98	6.5– 8.0	2.422	2.27	76.0	17.7	4.2	2.1
May 10	7a	4.64	4.0– 5.2	0.618	3.16	80.1	15.8	3.2	0.9
	b	5.54	5.0– 6.0	1.105	2.77	78.4	17.6	3.0	1.0
	c	6.45	5.8– 7.0	1.801	2.69	77.4	18.0	3.5	1.1
	d	7.26	6.6– 7.8	2.783	3.13	77.6	16.9	3.8	1.7
	e	8.06	7.6– 9.2	4.027	2.76	77.1	17.1	3.6	2.2
May 23	9a	5.50	5.2– 5.7	1.163	4.04	77.3	17.8	3.4	1.5
0745	b	5.92	5.6– 6.1	1.440	4.24	77.7	16.7	3.5	2.1
	c	6.24	6.1– 6.4	1.726	4.07	77.2	16.3	3.6	2.9
	d	6.80	6.4– 7.1	2.330	4.35	77.9	16.4	4.0	1.7
	e	7.16	6.8– 7.6	2.744	4.03	76.8	16.6	3.9	2.7

(*Continued*)

APPENDIX A. Sample statistics and percentage body composition of juvenile pink salmon, central British Columbia, 1963.

Date of sample 1963	Sample number	Mean fork length (*cm*)	Range of lengths (*cm*)	Mean live weight (g)	Stomach and contents (%)	Based on ex-stomach live weight			
						Moisture (%)	Protein (%)	Lipid (%)	Unknown (%)
May 23	10 a	5.85	5.3– 6.2	1.444	5.23	78.4	16.2	3.2	2.2
1045	b	6.38	6.0– 7.1	1.967	4.52	75.5	17.3	3.5	3.7
	c	7.02	6.5– 7.6	2.724	3.41	77.7	16.0	4.2	2.1
	d	7.75	7.1– 8.3	3.790	4.04	77.6	16.3	4.0	2.1
	e	8.72	8.0– 9.8	5.536	4.18	76.8	16.3	5.2	1.7
May 23	11a	5.88	5.3– 6.2	1.554	4.89	78.4	16.4	3.2	2.0
1345	b	6.48	6.3– 6.9	2.157	6.51	76.6	17.6	3.8	2.0
	c	7.10	6.7– 7.5	2.836	5.96	77.2	16.8	4.2	1.8
	d	7.62	7.2– 8.0	3.668	7.05	77.9	15.9	4.1	2.1
	e	8.38	7.5– 9.7	5.096	6.83	77.1	16.4	4.5	2.0
May 23	12a	6.82	5.9– 7.3	2.693	9.04	77.4	16.4	4.3	1.9
1645	b	7.62	7.2– 8.1	3.777	8.64	77.1	16.7	4.2	2.0
	c	8.11	7.5– 8.8	4.533	7.54	77.4	16.9	4.3	1.4
	d	8.52	8.2– 9.1	5.276	8.24	76.8	16.8	4.7	1.7
	e	9.34	8.8–10.2	6.902	6.98	76.4	17.9	4.3	1.4
May 23	13a	6.24	5.4– 6.7	1.934	8.22	79.6	15.0	3.5	1.9
1945	b	6.86	6.4– 7.2	2.566	7.36	79.9	16.1	3.8	0.2
	c	7.64	7.2– 8.3	3.777	6.55	78.3	16.4	3.8	1.5
	d	8.40	7.8– 9.1	4.854	6.75	7.77	15.9	4.6	1.8
	e	8.98	8.5– 9.9	6.079	6.06	76.8	17.6	4.5	1.1
May 23	14a	6.16	5.0– 6.7	1.834	6.84	78.6	17.0	3.6	0.8
2245	b	6.94	6.5– 7.4	2.704	6.40	77.9	16.6	4.3	1.2
	c	7.54	7.0– 8.0	3.488	6.34	75.5	17.5	4.4	2.6
	d	8.26	7.2– 8.9	4.721	6.01	77.4	17.0	4.7	0.9
	e	9.04	8.3–10.3	6.210	5.27	76.5	17.7	4.7	1.1
May 24	15a	5.77	4.6– 6.6	2.026	4.18	77.4	17.9	3.6	1.1
0145	b	6.64	5.9– 7.2	2.300	4.63	78.5	16.6	3.2	1.7
	c	7.22	6.5– 7.9	2.844	4.17	76.0	18.2	4.0	1.8
	d	8.04	7.2– 8.8	4.022	4.25	76.0	18.7	4.2	1.1
	e	8.94	8.3–12.1	6.164	4.72	75.8	17.8	5.0	1.4

(*Continued*)

APPENDIX A. Sample statistics and percentage body composition of juvenile pink salmon, central British Columbia, 1963. — (*Concluded*).

Date of sample 1963	Sample number	Mean fork length (*cm*)	Range of lengths (*cm*)	Mean live weight (*g*)	Stomach and contents (%)	Based on ex-stomach live weight			
						Moisture (%)	Protein (%)	Lipid (%)	Unknown (%)
May 24	16a	6.40	5.5– 6.9	1.910	3.74	78.8	16.4	3.8	1.0
0445	b	6.94	6.6– 7.4	2.510	3.57	77.6	16.6	4.0	1.8
	c	7.68	7.3– 8.1	3.497	2.97	76.3	18.3	4.2	1.2
	d	8.02	7.5– 8.5	4.173	3.15	76.8	17.7	5.0	0.5
	e	8.74	8.1– 9.1	5.440	2.72	76.3	17.8	4.8	1.1
May 29	17a	7.56	7.1– 8.0	3.754	5.58	77.0	17.4	4.4	1.2
0920	b	8.33	7.9– 8.5	4.986	5.34	75.9	18.7	4.3	1.1
	c	8.72	8.3– 9.1	5.574	5.34	74.6	19.3	4.6	1.5
	d	8.97	8.7– 9.3	6.279	5.49	75.0	18.7	5.6	0.7
	e	9.54	9.1–10.3	7.470	5.42	75.3	18.4	5.7	0.6
June 5	18a	8.74	8.3– 9.2	5.830	7.86	77.9	17.9	2.9	1.3
	b	9.44	9.2– 9.7	7.130	8.16	77.2	17.2	2.6	3.0
	c	9.76	9.4–10.2	7.938	8.16	77.5	20.0	2.3	0.2
	d	9.98	9.6–10.5	8.434	8.61	78.3	18.4	2.4	0.9
	e	10.51	10.1–11.0	10.186	8.40	77.3	18.9	2.5	1.3
June 11	19a	8.26	7.3– 8.8	4.204	2.72	76.6	20.0	2.9	0.5
0615	b	8.94	8.6– 9.2	5.430	2.62	77.6	18.4	2.6	1.4
	c	9.36	9.2– 9.8	6.260	2.72	78.4	18.5	2.2	0.9
	d	9.88	9.6–10.4	7.361	2.57	77.7	18.2	2.6	1.5
	e	10.54	9.9–11.8	8.992	2.64	77.4	19.5	2.8	0.3
June 11	20a	8.30	7.6– 8.8	4.284	3.43	77.9	18.2	2.4	1.5
1015	b	8.90	8.6– 9.1	5.382	3.06	76.6	19.1	2.7	1.6
	c	9.20	8.9– 9.5	6.026	3.38	78.0	17.4	2.7	1.9
	d	9.54	9.2–10.0	6.736	3.37	76.9	18.3	2.8	2.0
	e	10.22	9.7–11.5	8.624	3.52	77.4	18.3	2.7	1.6
June 11	21a	8.08	7.5– 8.4	4.028	5.09	78.1	17.6	3.0	1.3
1815	b	8.54	8.3– 8.8	4.864	5.93	76.1	19.2	3.1	1.6
	c	9.08	8.7– 9.6	6.012	5.55	77.0	18.6	3.0	1.4
	d	9.44	9.0– 9.8	6.609	5.39	77.2	18.8	2.6	1.3
	e	9.90	9.5–10.7	7.818	5.53	77.4	19.6	2.8	0.2

APPENDIX B. Sample statistics and percentage body composition of juvenile pink salmon, central British Columbia, 1964.

Date of sample 1964	Sample number	Mean fork length (*cm*)	Range of lengths (*cm*)	Mean live weight (g)	Stomach and contents[a] (%)	Based on total live weight (stomachs empty) Moisture (%)	Protein (%)	Lipid[b] (%)	Unknown (%)
April 15	1	3.84	3.5– 4.6	0.29	...[c]	84.4	11.0	3.3	1.3
May 3	10	4.52	3.7– 5.6	0.49	3.26	82.1	13.2	4.1	0.6
7	22	4.79	3.6– 6.4	0.60	8.21	82.4	13.2	4.3	0.1
12	32	5.24	4.2– 6.8	0.81	7.67	81.0	14.4	4.7	–0.1
14	42	5.61	4.8– 7.9	0.99	7.05	81.7	14.2	4.2	–0.1
22	60	6.82	3.7– 9.3	2.03	6.01	80.3	14.3	3.2	2.2
June 2	68	8.91	7.2–10.4	4.81	8.71	78.8	16.6	2.6	2.0
3	70	7.89	5.3– 9.9	3.49	5.98	80.5	14.5	1.8	3.2
10	124	8.99	7.6–10.4	4.97	2.92	79.2	17.0	1.3	2.5
10	125	8.04	7.3– 9.4	3.47	3.92	80.4	15.8	1.5	2.3

[a]Based on samples of 25 fish stored in 4% solutions of formaldehyde in sea water.
[b]Correction coefficient of 2.6 applied (see text).
[c]Stomach sample not available.

APPENDIX C. Sample statistics and percentage body composition of juvenile pink salmon held in live cage, 1963.

Date of sample 1963	Sample number	Mean fork length (*cm*)	Range of lengths (*cm*)	Mean live weight (g)	Stomach and contents (%)	Based on ex-stomach live weights			
						Moisture (%)	Protein (%)	Lipids (%)	Unknown (%)
April 28	2a	3.38	3.1–3.5	0.18	0.55	75.3	17.2	...[a]	...[a]
held	b	3.58	3.4–3.7	0.21	0.97	78.9	16.6	2.6	1.9
7 days	c	3.63	3.5–3.8	0.24	0.42	78.5	16.2	2.3	3.0
10,000/m³	d	3.90	3.6–4.2	0.30	0.33	78.0	17.1	...[a]	...[a]
	e	4.13	3.9–4.4	0.37	1.32	78.8	16.5	2.9	1.8
May 7 held	6a	3.63	3.4–3.8	0.26	1.53	81.2	15.1	2.0	1.7
9 days	b	3.81	3.6–4.0	0.31	1.63	82.4	13.6	1.8	2.2
additional	c	3.89	3.8–4.1	0.33	1.15	80.6	15.2	2.1	2.1
200/m³	d	3.95	3.8–4.1	0.33	1.21	80.5	16.0	1.9	1.6
	e	4.08	3.9–4.3	0.37	1.21	82.6	13.5	1.9	2.0
May 11 held	8a	3.70	3.5–3.9	0.26	1.67	sample missing			
4 days	b	3.84	3.7–4.0	0.30	2.01	81.0	14.2	1.8	3.0
additional	c	3.90	3.8–4.0	0.32	1.67	sample missing			
100/m³	d	3.94	3.8–4.0	0.33	1.92	81.3	14.6	2.3	1.8
	e	4.06	4.0–4.2	0.38	2.09	80.9	15.2	2.3	1.6

[a]Lipid determination was not achieved.

APPENDIX D. Sample statistics and percentage body composition of juvenile pink salmon held and starved in an aquarium, 1964.

Date of sample 1964	Sample number	Days held	Fork length (*cm*)	Range of lengths (*cm*)	Mean live weight (g)	Based on total live weight			
						Moisture (%)	Protein (%)	Lipid (%)	Unknown (%)
April 15	1	0.2	3.83	3.5–4.1	0.296	84.4	11.0	3.6	1.0
16	2	1.2	3.86	3.6–4.5	0.294	84.7	10.7	3.4	1.2
17	3	2.2	3.84	3.5–4.4	0.280	83.9	12.7	3.5	–0.1
20	4	3.5	3.90	3.6–4.2	0.274	86.2	10.3	2.6	0.9
25	5	8.2	3.84	3.5–4.6	0.241	88.9	8.2	1.7	1.2
May 5	6	18.2	3.82	3.5–4.1	0.210	87.4	9.5	1.5	1.6

Oxidative Rancidity in Frozen Stored Cod Fillets[1]

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ABSTRACT

During frozen storage at −18 and −25 C the lipids in cod muscle did not undergo oxidation, as indicated by thiobarbituric acid values and odours. In fact they underwent a marked decrease in the ease with which they were oxidized by added Cu^{++}, Fe^{++}, or hemoglobin. This change preceded the protein denaturation that occurs in stored frozen muscle and appeared to be directly related to the formation of free fatty acids in the muscle. A similar change in the sensitivity to metal-induced oxidations could be produced in fresh, unfrozen muscle by the addition of mixed fatty acids prepared from several marine lipids.

The addition of four pure saturated fatty acids had little or no effect on the development of rancidity in muscle, either in the presence or absence of added metal catalysts. Fish muscle appears to exert a protective action against the oxidation of added linolenic or linoleic acids. Unlike the mixed marine fatty acids, pure linoleic and linolenic acids did not suppress the development of metal-induced rancidities in fish muscle lipids.

INTRODUCTION

Under normal conditions fresh and frozen cod muscle rarely becomes rancid. At temperatures above freezing, bacterial activity creates conditions that are unfavourable to lipid oxidation (Lea, 1938; Lerke et al., 1961; Stansby, 1962; Patton, 1962; Castell and MacLean, 1964a, b). During frozen storage, where bacterial activity has been prevented, one must look for some other explanation.

Cod muscle has a lipid content ranging from 0.5 to 1%, and slightly higher in the lateral strip of dark muscle, but this relatively small concentration of lipid is not the explanation for the infrequency of rancidity. Under the right conditions, cod muscle can become intensely rancid (MacLean and Castell, 1964). It is more probable that the presence of natural antioxidants and the physical distribution of the lipids in the tissue are contributing factors. The composition of muscle is so complex and the relationship of the lipids to the nonlipids so intricate that one cannot predict reactions similar to those that occur in simpler systems.

This paper is concerned with rancidity in frozen cod muscle and some of the factors that affect its development, or lack of development. Of special interest are the two major changes that are known to occur in cod muscle during frozen storage: the accumulation of free fatty acids (FFA) resulting from lipid hydrolyses (Dyer and Morton, 1956), and the denaturation of some

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of the soluble proteins. These reactions are not independent of each other (Dyer and Fraser, 1959). The addition of FFA causes the actomyosin to become insoluble (King et al., 1962, Anderson and Steinberg, 1964). It has been further demonstrated that the unhydrolyzed phospholipids and neutral fats retard the denaturation of the protein brought about by the unsaturated fatty acids (Anderson and Steinberg, 1964; Hanson and Olley, 1965). In the literature on this subject, very little mention has been made of any oxidation taking place, either in the FFA or in the neutral fats during these changes in the frozen fish. Dyer et al. (1958) pointed out that as the lipids contain some highly unsaturated fatty acids, one would expect that the release of these acids in the tissues would increase the susceptibility of the muscle to rancidity. Apparently this is not so, and there is no clear explanation for the mechanisms involved. For this reason some experiments have been carried out with two objectives in view: (1) to determine the extent of lipid oxidation taking place in cod fillets during frozen storage, and (2) to find out whether the addition of free fatty acids or the denaturation of protein affects the course of lipid oxidation in the muscle.

Castell and MacLean (1964a, b) showed a considerable variation in the ease with which the cod muscle lipids can be oxidized by the addition of trace amounts of copper ions. It was found that one factor influencing the susceptibility of the muscle to Cu^{++}-induced rancidity was the time of the year in which the fish were caught, and is probably related to their spawning and feeding habits. The same procedures have been used to determine whether, during storage, changes take place in the frozen muscle affecting its susceptibility to metal-induced rancidity.

This work has been done with the hope that it might lead to an explanation of the apparent resistance of frozen cod muscle to rancidity, and to a definition of some of the conditions under which this resistance breaks down.

GENERAL PROCEDURES

Measurement of Rancidity

Rancidity has been measured by the thiobarbituric acid (TBA) reaction, using the distillation procedure of Tarladgis et al. (1960) and the reagents have been purified as suggested by Yu and Sinnhuber (1964). Details of the application of this test to cod muscle have been given in previous papers (MacLean and Castell, 1964; Castell et al., 1966b). When frozen fillets were being tested, they were always cut up into small pieces and put into the blender jar while still in the frozen state.

Susceptibility to Metal-Induced Rancidity

Although all samples of fish muscle will go rancid if sufficient Cu^{++} is added, there is considerable variation in the minimum amounts of these ions that are required to induce rancidity under a given set of conditions. In these studies it has been found convenient to estimate this relative sensitivity

to Cu^{++} by adding 20 ppm Cu^{++} to muscle (blended 1:3 with water), and then measuring the TBA value after 24 hr at 0 C (Castell and MacLean, 1964a, b). Muscle has been termed "susceptible" when it develops strong seaweedy or rancid odours and TBA values above 0.30 within 24 hr after adding 20 ppm Cu^{++} to the blend; conversely an unsusceptible muscle is one from which no seaweedy or rancid odours are produced and the TBA values remain below 0.15 under these same conditions. The degree of susceptibility is purely relative.

Free Fatty Acids

The determination of total free fatty acids (FFA) in the muscle was carried out by the procedure used by Bligh and Dyer (1959). The results are expressed as percentage of FFA in the extracted lipids, and have been calculated as oleic acid. The fatty acids that were added to the muscle came from several sources. Most of the pure oleic, linoleic, and linolenic acids were obtained from Hormel Institute; pure linoleic acid was also obtained from Nutritional Biochemicals Corporation and from Mann Laboratories. Four pure saturated fatty acids were prepared by Dr R. G. Ackman and his associates at this laboratory. They also prepared the natural mixed fatty acids from cod liver oil and seal blubber. The unsaturated fatty acids and the mixed fatty acids from marine sources were kept in sealed glass containers and then handled and transferred under nitrogen using a micro-syringe with a long needle.

Extractable Proteins

Denaturation of the muscle was followed by measuring the changes in extractable protein (Dyer et al., 1950; Snow, 1950).

Frozen Storage

For the longer storage periods at –25 and –18 C good quality fillets were cut from gutted, trawler-caught cod, less than 4 days on ice. The fillets were packed in 1-lb waxed boxes and frozen in a commercial plate-freezer. These in turn were placed in corrugated cardboard boxes overwrapped with waxed paper and sealed with scotch tape. For shorter storage periods at –3 C the fillets were individually frozen in sealed, laminated polyethylene bags, which had an outer layer of cellophane bonded to an inner layer of polyethylene. As will be noted, some were frozen in a plate-freezer and others were frozen by exposing them to the atmosphere of a cold-storage room (–25 C) for 24 hr prior to storage at –3 C.

RANCIDITY IN STORED FILLETS

Tests were carried out to determine whether the general observation that "frozen cod fillets are resistant to oxidative rancidity" could be confirmed by experiment. Fillets were frozen and stored at various temperatures

and periodic measurements were made to follow any oxidation that occurred. None of the fish stored at −12, −18, or −25 C became rancid during the storage periods that were used. The following are typical:

Fillets cut from winter-caught cod were frozen and stored for 1290 days at −25 C. They developed no seaweedy or rancid odours and no other odours suggesting oxidative rancidity. The TBA values (Table I) also showed that little or no oxidation of the lipids had taken place.

Figures 1 and 2 show the periodic TBA values for three lots of fish stored at −12, −18, and −25 C. Although these storage periods are relatively short (132 and 100 days), it can be seen by inspection that there is no increase in the TBA values at −18 or −25 C, but a definite trend upwards at −12 C.

TABLE I. TBA values for one lot of frozen cod fillets during 1290 days of frozen storage at −25 C.

Storage period (*days*)	TBA values
0	0.02
7	0.02
18	0.02
102	0.03
160	0.03
210	0.05
420	0.06
1290	0.05

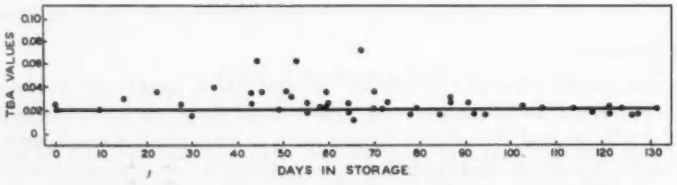

FIG. 1. TBA values of frozen cod fillets held for 132 days at −25 C.

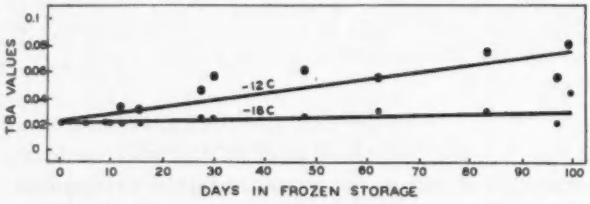

FIG. 2. TBA values of frozen cod fillets held for 100 days at −12 and −18 C.

In two other series of tests at −18 and −25 C no increase in TBA values was obtained during 270 days of storage.

Twelve lots of fillets in waxed boxes, overwrapped with waxed paper, were stored for various periods ranging from 98 to 210 days at −18 C. Initial mean TBA values from five fillets from each lot are given in the first column of Table II and the corresponding values at the end of the storage periods are given in the second column. These TBA values and the entire absence of any odours suggesting rancidity indicate that little or no oxidation of the lipids took place.

Commercially-packed cod fillets stored for 1 year in 1-lb waxed, overwrapped boxes, in a commercial cold-storage room intended to be at about −25 C (but sometimes fluctuating as much as 5 C degrees) had initial TBA values of 0.02 and a mean value of 0.041 (for fillets from five boxes) at the end of the storage period.

Commercial fillets in waxed cartons, overwrapped with coated cellophane, were stored in a cold room (−25 C) for various lengths of time. The TBA values (in parentheses) at the end of the storage periods were as follows: months stored, 4(0.03); 7(0.04); 14(0.05); 20(0.05); 43(0.05). Fillets stored for 14–43 months showed some deterioration in appearance but did not smell rancid.

In a few instances cod fillets with high TBA values and rancid odours were encountered. They had been frozen in waxed cartons, but not overwrapped, and stored for 7 months in a room at −9 to −11 C. At the end of the storage period they showed considerable dehydration and discolouration and had odours that ranged from "old dried salt fish" odour to that of "rancid fish oil." TBA values ranged from 0.38 to 0.61. Those that were most dehydrated had the highest TBA values and the most rancid odours.

TABLE II. Changes in TBA value and susceptibility to copper-induced rancidity for 12 lots of frozen cod fillets before and after storage at −18 C.

Initial TBA values	TBA values after storage	Storage period (*weeks*)	Sensitivity to copper (TBA values)	
			Initial	After storage
0.02	0.03	23	0.63	0.05
0.02	0.05	23	0.57	0.07
0.03	0.04	30	0.47	0.04
0.02	0.05	23	0.46	0.20
0.02	0.04	14	0.37	0.06
0.02	0.03	21	0.26	0.06
0.01	0.04	14	0.24	0.04
0.02	0.05	23	0.23	0.06
0.02	0.03	18	0.22	0.13
0.02	0.03	18	0.16	0.06
0.03	0.07	22	0.09	0.12
0.02	0.03	18	0.05	0.03

CHANGES IN SUSCEPTIBILITY TO METAL-INDUCED RANCIDITY DURING FROZEN STORAGE

By making periodic tests on fillets in cold storage it was found that they underwent a marked decrease in the ease with which they went rancid following the addition of 20 ppm Cu^{++} or Fe^{++}. The rate of change was dependent upon the storage temperature (Fig. 3).

Fish caught during the late spring and summer months are usually relatively insensitive to added Cu^{++} (Castell and MacLean, 1964a, b). For this reason the change in sensitivity to added copper during frozen storage is less obvious with these initially insensitive fish. This can be seen by comparing the results from the 12 lots of fillets in the last two columns of Table II. These were purposely arranged in order of their initial sensitivity to added Cu^{++}.

Tests such as those indicated in Fig. 3 were repeated several times and the curves in each case were approximately the same.

CHANGES IN SUSCEPTIBILITY TO HEMATIN-INDUCED RANCIDITY DURING FROZEN STORAGE

It was found that Fe^{++} as well as Cu^{++} became less effective as a catalyst of oxidative rancidity as the storage time for frozen cod fillets was prolonged. Neither of these metals, however, is likely to be present as free ions in sufficient concentrations to be the normal catalysts of rancidity in fish muscle. It is

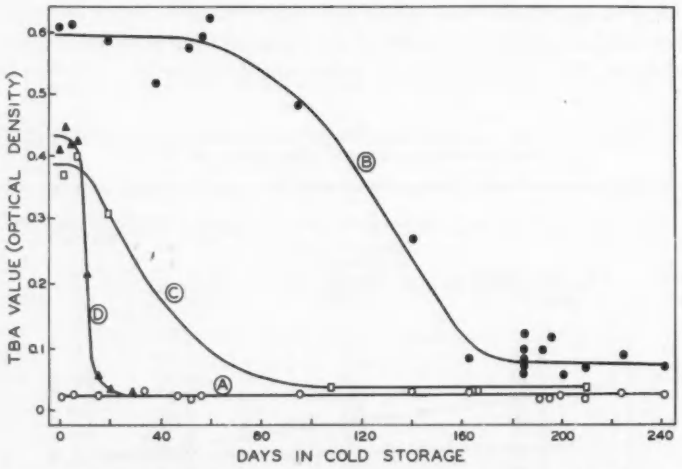

FIG. 3. A — Effect of storage period on the development of rancidity in cod fillets stored at −25 C as indicated by TBA values. B — Changes in the sensitivity of the frozen stored (−25 C) fillets to Cu^{++}-induced rancidity as shown by TBA values 24 hr after adding 20 ppm Cu to the blended muscle. C and D — Changes in the sensitivity to added Cu^{++} of fillets stored at −18 and −3 C, respectively.

much more likely that organic iron in the form of hematin compounds is involved (Brown et al., 1957; Stansby and Brown, 1958; Tappel, 1962). The addition of 500 ppm of hemoglobin to blended fresh cod muscle consistently produced high TBA values and "fishy, rancid" off-odours within 24 hr at 0 C. Similar tests were made with stored frozen fillets that had become relatively insensitive to added Cu^{++} and Fe^{++}. The results showed that they had also become very much less sensitive to the catalytic effect of added hemoglobin (Table III).

TABLE III. Comparison of the sensitivity of fresh and frozen stored cod fillets to added Cu^{++} (20 ppm) and hemoglobin (500 ppm) as indicated by TBA values and odours.

	Effect of added Cu^{++}		Effect of hemoglobin	
Fillet	TBA values	Odours	TBA values	Odours
Fresh	0.525	Rancid	0.415	Fishy-rancid
Fresh	0.580	Rancid	0.595	Fishy-rancid
Fresh	0.600	Rancid	0.570	Fishy-rancid
Frozen-stored	0.200	Slightly seaweedy	0.120	Not much odour
Frozen-stored	0.135	Not much odour	0.120	Not much odour
Frozen-stored	0.035	Not much odour	0.055	Not much odour

EFFECT OF FREEZING TEMPERATURE ON THE SUBSEQUENT SENSITIVITY OF THE MUSCLE TO CU^{++}

The rate of freezing had a pronounced effect on the subsequent reaction of the muscle to added Cu^{++} and Fe^{++}. This is shown by the following experiment. Similar fillets were sealed in laminated, polyethylene bags and divided into five lots for different freezing treatments. Following freezing they were stored at −3 C for 24 hr and then tested for sensitivity to added copper and the development of copper-induced odours:

Freezing treatment	*TBA value*	*Odour*
Directly into room at −3 C	0.530	seaweedy-rancid
1 hr in plate freezer at −40 C	0.535	seaweedy-rancid
24 hr in room at −25 C	0.475	strong seaweedy
24 hr in room at −15 C	0.145	slightly seaweedy
24 hr in room at −13 C	0.105	slightly seaweedy

These results were confirmed by other tests, including those in which the fillets were rapidly frozen at −60 C. Rapid, low-temperature freezing

resulted in less decrease in sensitivity of the muscle to Cu^{++}-induced rancidity. There are two factors, therefore, that must be considered in studying the changes in the ease by which frozen muscle is oxidized by added Cu^{++} or Fe^{++}: the rate at which the fish is frozen and the subsequent temperature at which it is stored.

Effect of Free Fatty Acids on Rancidity

Experiments dealing with the effect of fatty acids on the development of rancidity were of two types: those in which the FFA were formed naturally as a result of lipid hydrolysis during storage; and other experiments in which the fatty acids were added directly to blended muscle. Under these two conditions there is a considerable difference in the physical distribution of the FFA in relation to other components of the muscle with which they may react (Hanson and Olley, 1965).

Naturally Formed FFA

Two lots of similar cod fillets were frozen in plastic bags, stored at −3 C for different storage periods, and then tested for FFA and for sensitivity to added copper. The results showed (Fig. 4) that as the FFA increased, the

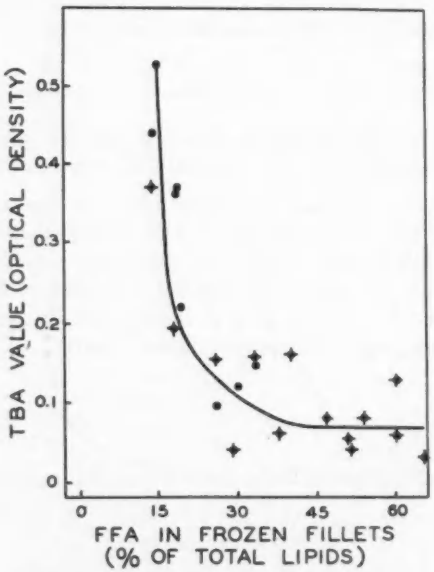

Fig. 4. Relation between free fatty acid content in frozen cod fillets and their susceptibility to Cu^{++}-induced rancidity. These values are from two lots of fillets held in storage for various periods of time.

sensitivity of the muscle to added Cu^{++} decreased, up to an FFA value of approximately 35–40%. At this level of FFA and higher, the addition of 20 ppm Cu^{++} ceased to affect the TBA values of the blended muscle during the 24-hr holding period. FFA determinations were also made on 53 fillets that had been frozen under different conditions and stored for various periods of time, giving FFA values varying from 9 to 71%. Each fillet was also tested for its TBA value and its sensitivity to added Cu^{++}. There was no increase in the TBA values of the frozen fillets as the FFA increased (Fig. 5), but there was a sharp decrease in the sensitivity of the muscle to added Cu^{++} and a curve obtained was quite similar to that of the preceding experiment.

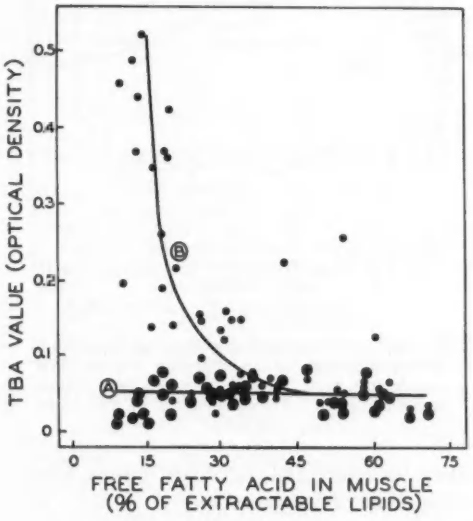

FIG. 5. The relation between free fatty acids in frozen cod fillets and TBA values (A) and susceptibility to copper-induced rancidity (B) as indicated by TBA values 24 hr after adding 20 ppm Cu^{++} to the blended muscle. These values are from many different lots of fillets frozen and stored under a variety of conditions.

MIXED FFA FROM MARINE LIPIDS

The addition[2] of natural mixed FFA prepared from freshly extracted cod liver oil and seal blubber also decreased the sensitivity of blended cod muscle to rancidity induced by Fe^{++}, Cu^{++}, and hemoglobin (Fig. 6 and 7). In addition

[2]Where free fatty acids have been added to the blended muscle the concentrations are recorded as per cent FFA in the blend, which is one part muscle and three parts water. Thus, 0.1% in the blend is equal to 0.4% in the muscle. Since cod muscle contains approximately 0.8% lipid, 0.1% FFA added to the blend roughly equals 50% of its normal lipid content.

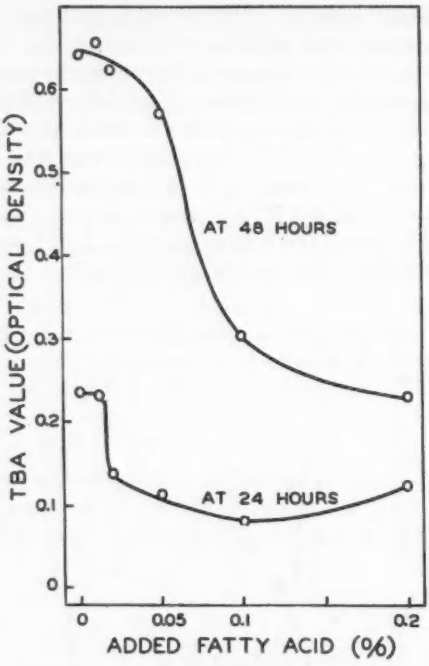

FIG. 6. Effect of adding seal oil fatty acids on the development of rancidity in blended cod muscle to which 20 ppm Cu^{++} had been added as indicated by TBA values after 24 and 48 hr at 0 C.

to retarding rancidity, as indicated by TBA values, these mixed FFA also suppressed the development of rancid odours.

ADDITION OF PURE FATTY ACIDS

It seemed likely that the suppression of metal-induced rancidity by the fatty acids would be caused by either their double bonds or by their carboxyl groups. Because of the difficulty of getting the available saturated fatty acids into solution at 0 C, tests were carried out with four dicarboxylic acids (succinic, malonic, adipic, and azelaic acids). They did not suppress the Cu^{++}-induced rancidity in the fish muscle (Table IV), suggesting that the inhibition was not the result of carboxyl groups. This was later confirmed by the fact that almost identical results were obtained with linoleic acid and methyl linoleate.

Unsaturated fatty acids (linoleic and/or linolenic) from four different sources were used. The first was linoleic and prepared in our marine oils laboratory by Dr Ackman and his associates. Tetrabromostearic acid was debrominated and then distilled and the proper fractions selected to give relatively

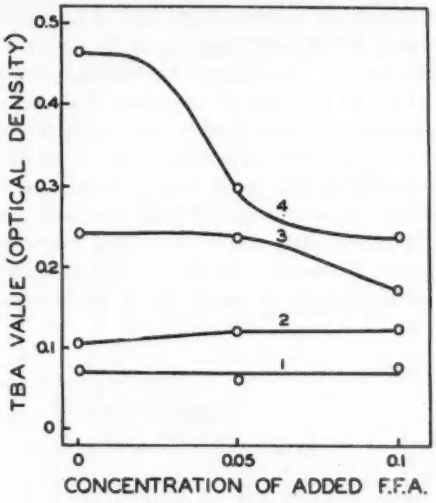

FIG. 7. Inhibiting effect of cod liver oil fatty acids on hemoglobin-induced rancidity in blended cod muscle stored at 0 C for 24 hr. 1. Control — no hemoglobin; 2. 100 ppm hemoglobin; 3. 200 ppm hemoglobin; 4. 500 ppm hemoglobin.

TABLE IV. The effect of adding four saturated fatty acids on the development of rancidity in blended cod muscle at 0 C with and without the addition of 20 ppm Cu^{++}.

Acid added (to give 0.2%)	Cu^{++} added (to give 20 ppm)	Odour (24 hr)	TBA values 24 hr	TBA values 48 hr
None	–	Not much odour	0.02	0.02
Succinic	–	Not much odour	0.05	–
Malonic	–	Not much odour	0.03	–
Adipic	–	Not much odour	0.02	–
Azelaic	–	Not much odour	0.02	–
None	+	Rancid	0.62	0.67
Succinic	+	Rancid	0.50	0.60
Malonic	+	Rancid	0.55	0.63
Adipic	+	Rancid	0.56	0.66
Azelaic	+	Rancid	0.56	0.66

pure linoleic acid. Tests carried out with this sample gave results similar to those obtained with the mixed marine FFA, i.e. when added to the blended muscle to give concentrations of 0.05, 0.1, and 0.2% it inhibited or retarded copper-induced rancidity. Pure linolenic and/or linoleic acids were obtained from Hormel Institute, Mann Laboratories, and Nutritional Biochemical Corporation. During the months of September to January, inclusive, a series

of 17 experiments was carried out in which 0.05, 0.1, and 0.2% of these acids were added to blended fresh cod muscle, with and without the addition of 20 ppm Cu^{++}. Instead of suppressing metal-induced rancidity these fatty acids accelerated it (Fig. 8). In a few of the tests, the results suggested that in the lowest concentrations used the linoleic acid might have slight inhibiting action (Fig. 8 and 10) but did not always occur. In order to make sure that the difference was not in the muscle itself, pure linoleic acid and cod liver oil fatty acids were added to blends prepared from the same fillet (Fig. 10). Again the pure linoleic acid accelerated while the mixed fatty acids depressed the Cu^{++}-induced rancidity. However, as the "natural" fatty acids in muscle increased during frozen storage the accelerating effect of added pure linoleic acid decreased (Fig. 9).

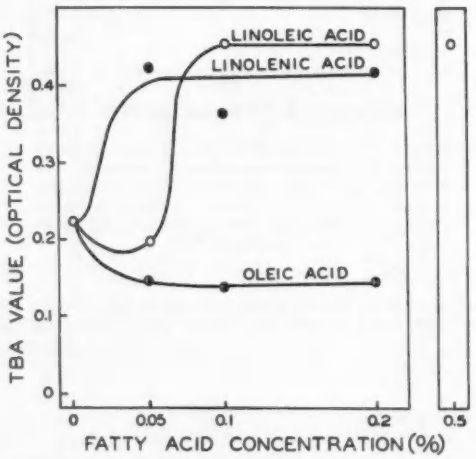

FIG. 8. Effect of adding 0, 0.05, 0.1, 0.2% oleic, linoleic and linolenic (and 0.5% linoleic) acids on the development of Cu^{++}-induced rancidity in blended cod muscle after 24 hr at 0 C.

In the absence of metal catalysts the addition of unsaturated fatty acids to the muscle had surprisingly small effect on the subsequent TBA values. For example, linoleic acid was added to both water and to blended fresh cod muscle to give a concentration of 0.1%. Within 5 min the TBA value of the aqueous suspension was 0.175 and continued to rise rapidly. After 30 min there was no change in the TBA value of the muscle to which the acid had been added; after 24 and 48 hr at 0 C it increased from an initial 0.025 to 0.04 and 0.055, respectively. In another series of tests linoleic acid was added to 10 different samples of freshly blended cod muscle to give concentrations of 0.1%. After 24 hr at 0 C the increased TBA values, over the corresponding controls without added acid, were 0.04, 0.01, 0.015, 0.01, 0.03, 0.00, 0.03, 0.02, 0.025,

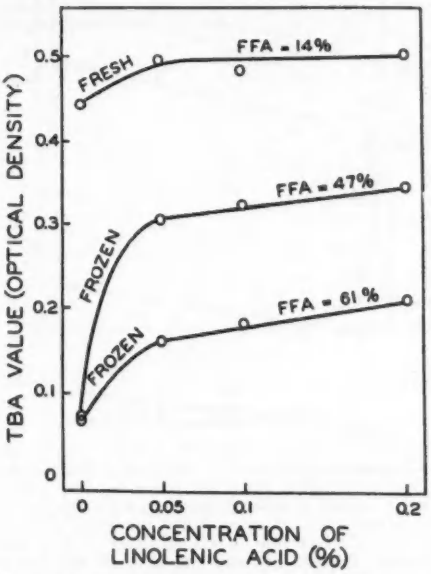

FIG. 9. Effect of pure linoleic acid (Hormel Inst.) on copper-induced rancidity in blended muscle prepared from fresh cod fillets and from similar fillets that had been held in frozen storage until their FFA content had increased to 47 and 61%.

and 0.015. This is an average increase of 0.02 in TBA values and does not represent very active oxidation. Similar tests with linolenic acid, cod liver oil fatty acids, and seal oil fatty acids gave average increases of 0.082, 0.01, and 0.15, respectively. Tests were also carried out to determine the effect of the concentration of added fatty acids on the development of rancidity (see control curves in Fig. 10). When the concentrations were 0.1% or less, with linoleic acid and the mixed fatty acids from the marine oils, the TBA values remained very small after 24 and 48 hr. The addition of 0.3 and 0.5% of these acids sometimes but not always raised the TBA values of the muscle to 0.15–0.25. Linolenic acid was much more susceptible: the upward curve in the TBA values began with the addition of 0.05–0.1%; 0.3 and 0.5% gave values between 0.27 and 0.42 and slightly rancid odour after 24 and 48 hr at 0 C. These results suggest that in the lower concentrations the fatty acids react with some component of the muscle, in a manner that protects them against oxidation.

RELATION TO PROTEIN DENATURATION

Tests were carried out to compare the rate of change in the sensitivity to Cu^{++}-induced rancidity with the rate of protein denaturation. The results

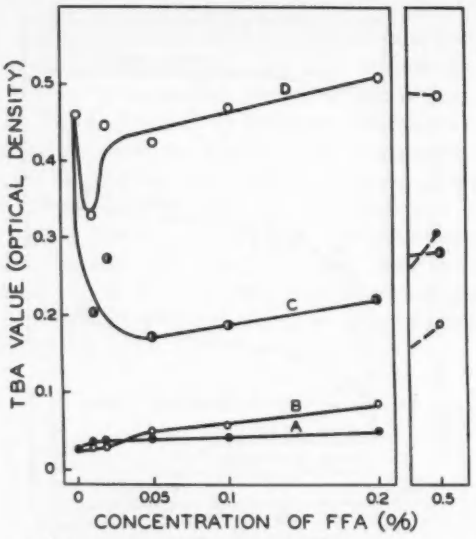

FIG. 10. Comparison of the effect of adding pure (Hormel) linoleic acid and cod liver oil fatty acids on the development of rancidity in blended cod muscle prepared from the same fillet and stored at 0 C. A — Linoleic acid. B — Cod liver oil fatty acids without added Cu^{++}. C — Cod liver oil fatty acids. D — Linoleic acid with 20 ppm Cu^{++} also added to the blended muscle.

showed that in each instance protein denaturation began after the change in the sensitivity of the lipids to added Cu^{++} had reached the minimum values. Treatments of the fish during freezing that accelerated the subsequent change in the ease of oxidation by added Cu^{++} also brought a corresponding acceleration in the rate of protein denaturation (Fig. 11).

DISCUSSION OF RESULTS

These results have confirmed the general observation that during normal frozen storage the lipids in cod fillets do not appear to undergo oxidative rancidity (Dyer and Fraser, 1959; Dyer et al., 1958). On the contrary, during frozen storage the muscle undergoes changes rendering it less susceptible to both metal-ion and hematin-catalyzed rancidity. This does not mean that cod muscle cannot become rancid during storage. If it is allowed to become dehydrated, or if strong oxidation catalysts are added to the muscle before freezing, rancidity will take place. But under normal conditions these things do not occur and therefore the fish do not become rancid.

Because the frozen muscle undergoes changes that decrease its susceptibility to rancidity, this suggests that the most critical period for initiating rancidity would be before the fish are frozen and for a relatively short time

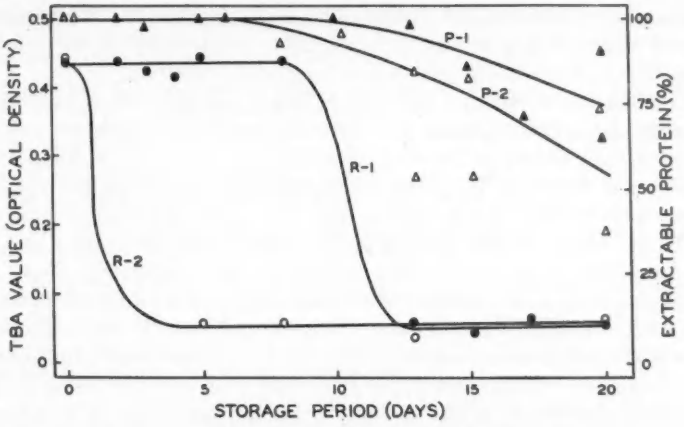

FIG. 11. Changes in two lots of cod fillets during frozen storage at −3 C. P-1 and P-2 indicate the change in extractable proteins and R1 and R2 show the corresponding changes in the sensitivity of the lipids to Cu^{++}-induced rancidity as indicated by TBA values 24 hr after adding 20 ppm Cu^{++}. The data points for each day represent values obtained from one or two whole fillets.

after they have gone into frozen storage. However, any tendency towards rancidity before freezing will only occur if bacterial activity has been held in check (Castell and MacLean, 1964a, b).

There appears to be a direct relationship between the decrease in the ease with which the lipid in the frozen muscle can be oxidized and the production of free fatty acids in the muscle. It was also shown that a similar decrease in the ease of oxidation in fresh unfrozen muscle can be brought about by the addition of mixed fatty acids obtained by hydrolyzing lipids from freshly prepared cod liver oil and from seal blubber. The addition of pure oleic acid also had a retarding action on metal-induced rancidity. The results with pure linolenic and linoleic acids usually resulted in accelerated rancidity in the presence of metal catalysts rather than inhibition. No explanation is forthcoming for this apparent contradiction in results with these pure unsaturated fatty acids.

The change in the sensitivity of the muscle to added catalysts occurred in the frozen fish when the FFA reached levels of 20–30% of the total lipids in the muscle. The same change occurred in the unfrozen muscle when the mixed marine fatty acids were added to give concentrations corresponding to 10–30% of the lipids normally present.

Linoleic and linolenic acids rapidly oxidize when exposed to the air or when prepared as aqueous suspensions. It was interesting, therefore, to observe how slowly these unsaturated acids oxidized when incorporated in the fish muscle in the absence of added catalysts. In concentrations of less than 0.1% they did not produce rancid odours and raised the TBA values of the muscle very slowly. Higher concentrations, especially with linolenic acid, did result in

rancidity and increased TBA values. This suggests that in low concentrations they may react with proteins or some other component of the muscle in a manner that protects their double bonds against oxidation.

Although the muscle of lean fish is much less subject to rancidity than the muscle of the fatty species, this does not mean that lipids are not directly involved in their deterioration. For all practical purposes the storage limitation of frozen cod fillets is the result of toughening and other texture changes resulting from protein denaturation. But it has been shown that this denaturation is the result of the interaction of unsaturated fatty acids and certain muscle proteins (King et al., 1962; Anderson and Steinberg, 1964; Hanson and Olley, 1965). These workers have also shown that neutral lipids in the muscle have a sparing effect on this reaction, and that for each species of fish there is a critical concentration of fatty acids beyond which the extractable protein decreases rapidly. From this it follows that the composition and concentration of the muscle lipids, and any reactions that take place in the lipids, may ultimately affect protein denaturation. Hydrolysis of the muscle phospholipids is of prime importance as being the chief source of the free fatty acids that are formed during storage. This raises the question of the significance of the change that occurs in the sensitivity of the muscle to rancidity to other changes that occur during frozen storage. For this we have no immediate answer. It is of interest, however, to note the chronological relationship between these reactions. Formation of free fatty acids was accompanied by a decrease in the sensitivity of the lipids to metal-catalyzed oxidation. This decrease in sensitivity of the lipids to oxidation reached a minimum level when the FFA content was 20–30% of the total lipids. It was not until after this point had been reached that further fatty acid formation was accompanied by the first indications of protein denaturation. Dyer and Fraser (1959) pointed out that protein denaturation began when the FFA had reached approximately 50% of the total lipids.

As the changes that occur in the lipids and proteins of frozen fish muscle are interrelated the results of tests carried out at −3 C may have some practical application. It is quite possible that these values that can be obtained in a few weeks might be useful in predicting the storage time of the much longer periods at lower temperatures. The procedure of following the changes occurring in the lipids of fish stored at −3 C might also provide a quicker method for comparing the keeping time of fish caught at different seasons of the year or stored and processed under different conditions before freezing. Although it is the protein denaturation that limits the keeping time of the frozen fish in storage, it is the change in the lipids that triggers the denaturation.

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Times of Annulus Formation on Scales of Sockeye, Pink, and Chum Salmon in the Gulf of Alaska[1]

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ABSTRACT

On scales of pink (*Oncorhynchus gorbuscha*), chum (*O. keta*), and possibly sockeye (*O. nerka*) salmon caught by Canadian research vessels throughout the Gulf of Alaska at various times of the year, the annual ring began to form sometime between early November and January. Many of the sockeye and pink salmon had completed the annulus sometime in December or January, whereas the chum salmon completed their's later, in February or March. For both sockeye and chum salmon, scales of younger fish tended to commence new growth at an earlier date than did those of older fish.

INTRODUCTION

This paper concerns the time of annulus formation for sockeye (*Oncorhynchus nerka*), pink (*O. gorbuscha*), and chum salmon (*O. keta*) taken on the high seas in the Gulf of Alaska from 1963 to 1965. Studies of age and growth of salmon require relatively precise estimates of the time of annulus formation on the scales. Until recently, information on the high-seas distribution of salmon was sparse, and in consequence there has been insufficient information on which to make appropriate studies.

Scales of Pacific salmon are roughly oval. Their outer surface bears a series of circular corrugations (circuli or sclerites) confined mainly to the anterior part of the scale. Bands of circuli are alternately widely spaced and packed closely together, the groupings being considered to reflect changes in growth throughout the year. The widely spaced bands of circuli presumably reflect rapid growth in the summer, and the compactly spaced bands of circuli slow growth in the winter. These bands have been given different names, which has caused some confusion in the literature. Chugunova (1959) called the part of a scale formed during one year (including both the band of widely spaced and that of compact circuli) an "annual zone" of growth of the scale; the boundary between the crowded autumn sclerites and the well-spaced spring–summer sclerites was termed an "annulus" or "year ring" or "annual ring." Gilbert (1922) called the band of narrowly spaced circuli the "winter check" and the part of the scale formed immediately after the winter check "new growth." Kobayashi (1959) called the band of narrowly spaced circuli the "resting zone," whereas Birman (1960), Semko (1954), and Vedensky (1954) called this band on the scale the "annual ring." Ricker (1962, 1964) used the term "annulus" to mean the outer edge of the last circulus of the compact band of circuli.

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Beckman (1943) designated the last single circulus in the band as the "annulus," which is similar to Chugunova's and Ricker's definition of the annulus. In this paper, a band of closely spaced circuli is called the "annual ring." The annulus is here defined as the last narrowly spaced circulus of the annual ring preceding the first, more widely spaced, circulus referred to here as the start of "new" growth.

The exact time of oceanic annulus formation is not known. Gilbert (1922) noted that scales of chum salmon taken in the Yukon River early in July had barely begun new growth and on some no new growth was evident. He concluded that the annual ring had formed in late winter or in early spring. Kobayashi (1959) examined scales from chum salmon collected in the North Pacific Ocean during May and early June 1956. Scales from many individuals showed no evidence of new growth with some still forming the annual ring. Scales from other individuals showed from one to four new growth circuli beyond the annual ring. Vedensky (1954) considered that pinks do not grow during the winter and that the annual ring forms either before the cessation of growth or, more likely, in the spring when the fish, weakened by winter hunger, begins feeding. Semko (1954) also suggested that the annual ring of pinks is formed in the spring, probably sometime in March.

Birman (1960) provides one of the most recent studies of the period of formation of the annual ring on the scales of Pacific salmon. He found that in April (1959) pinks which had spent the winter south of the Aleutians had from 7 to 13 new growth circuli beyond the annual ring. Using this information along with information on size, he suggested that the annual ring is completed by the beginning of winter, not later than the first half of January. For sockeye, captured in April, he found that most individuals in their fourth and fifth years had one or more circuli beyond the annual ring, but in some fish the annual ring had only just begun to form and in others it was not present. Many chums sampled in April still did not have an annual ring at the edge; in others it was only partially formed. In three instances new growth had only just begun. Birman concludes that, in general, pinks complete the annual ring in early January, sockeye apparently in March, and chums in April.

MATERIALS AND METHODS

Recently, scale samples became available from sockeye, pink, and chum salmon taken by Canadian research vessels in the Gulf of Alaska in January and April of 1964 and 1965, and in Hecate Strait, Chatham Sound, and Dixon Entrance in November 1963 (Fig. 1). These were examined to provide information about the time of annulus formation in these three species of salmon in these regions.

A total of 853 sockeye, 215 pink, and 133 chum scales was examined under a microprojector at 100 magnifications. The age of each fish and the number of circuli beyond the last annulus were recorded.

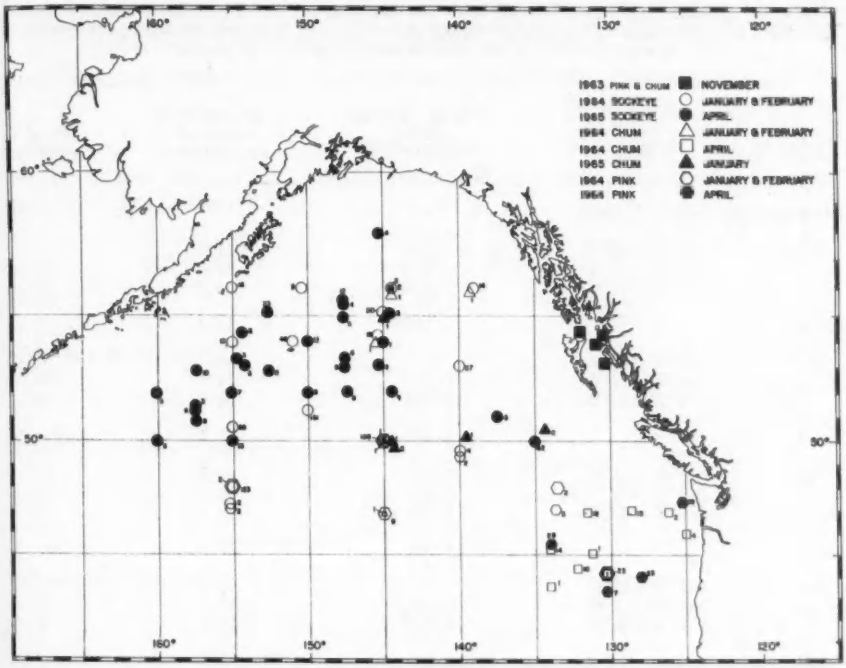

FIG. 1. Locations of the samples of sockeye, pink, and chum salmon from Canadian research vessels and coastal fisheries catches in the Gulf of Alaska, Dixon Entrance, Hecate Strait, and Chatham Sound, 1963–65.

Scales were also available from 300 mature pink salmon taken in coastal fisheries of British Columbia in the summer of 1964, and from 1795 mature chum salmon of the 1954 brood year taken in coastal fisheries of British Columbia and southeastern Alaska.

RESULTS AND DISCUSSION

SOCKEYE SALMON

The scales of 619 age 4_2, 5_2, 5_3, and 6_3 sockeye salmon[2] caught in the Gulf of Alaska from January 7 to February 7, 1964 (Fig. 1), indicated that for many the annulus had formed during the early part of the winter (Fig. 2 and Table I). Many of the scales already showed new growth, the proportion

[2]In the present paper, the Gilbert (1913) system of age designation is used. Here, age refers to the year of life dating from time of egg deposition rather than the number of anniversaries passed. For example, a sockeye captured in the fishery during the summer of 1964 that had arisen from eggs deposited in the autumn of 1960 would be in its fourth year of life and would be called a 4-year-old (4) even though it had only passed three anniversaries since egg deposition. The year in which migration to sea took place is indicated by a subscript. Thus, if the fish spent 1 year in fresh water before migrating to sea in its second year, the age of the particular fish would be designated as 4_2.

TABLE I. Average numbers of circuli after the last annulus on the scales of sockeye, pink, and chum salmon caught in the Gulf of Alaska during January and April 1964 and 1965. (Scales lacking circuli after the annulus are included in the means.)

Age	Area	Average number circuli after annulus	Per cent with no circuli after annulus	Total samples
Sockeye (Jan. 7–Feb. 7, 1964)				
4_2	135°W–143°W	2.653	11.5	26
	144°W–151°W	1.874	31.1	103
	152°W–160°W	1.626	34.3	99
Total:		1.855	30.3	228
5_2	135°W–143°W	0	0	2
	144°W–151°W	0.815	44.5	200
	152°W–160°W	0.956	34.8	46
Total:		0.855	42.3	248
5_3	135°W–143°W	1.917	16.7	12
	144°W–151°W	1.853	32.3	34
	152°W–160°W	1.091	45.4	55
Total:		1.446	37.6	101
6_3	135°W–143°W	...	...	0
	144°W–151°W	0.576	57.6	33
	152°W–160°W	1.111	44.4	9
Total:		0.690	54.8	42
Sockeye (Apr. 4–28, 1965)				
4_2	135°W–143°W	4.708	0	48
	144°W–151°W	4.700	0	50
	152°W–160°W	...	...	0
Total:		4.704	0	98
5_2	135°W–143°W	...	...	0
	144°W–151°W	2.562	0	32
	152°W–160°W	2.888	0	18
Total:		2.680	0	50
5_3	135°W–143°W	...	...	0
	144°W–151°W	...	...	0
	152°W–160°W	4.500	0	46
Total:		4.500	0	46
6_3	135°W–143°W	...	...	0
	144°W–151°W	...	...	0
	152°W–160°W	2.625	0	40
Total:		2.625	0	40

(*Continued*)

TABLE I. Average numbers of circuli after the last annulus on the scales of sockeye, pink, and chum salmon caught in the Gulf of Alaska during January and April 1964 and 1965. (Scales lacking circuli after the annulus are included in the means.) — (*Concluded*)

Age	Area	Average number circuli after annulus	Per cent with no circuli after annulus	Total samples
Pink (Jan. 7–Feb. 7, 1964)				
2_1	133°W–140°W	1.500	25.0	8
	141°W–155°W	1.867	20.0	15
Total:		1.739	21.7	23
Pink (Apr. 12–27, 1964)				
2_1	–	6.649	0	97
Chum (Jan. 7–Feb. 7, 1964)				
3_1	–	0	100	6
4_1	–	0	100	2
4_1	–	0	100	6
Chum (Apr. 12–27, 1964)				
3_1	–	5.342	0	35
4_1	–	4.421	0	19

TABLE II. Average number of circuli on the scales of pink and chum salmon caught in Hecate Strait, Dixon Entrance, and Chatham Sound in November 1963.

Location	Time	Species	Average number circuli to edge of scale	Average number circuli to annulus	Per cent with start of annual ring at edge of scale	Total samples
Hecate Strait, Dixon Entrance, Chatham Sound	Early November 1963	Pink	18.8		30.5	95
Shore areas, British Columbia 1964		Pink		26.2		300
Hecate Strait, Dixon Entrance, Chatham Sound	Early November 1963	Chum	22.9		29.2	65
Shore areas, British Columbia, southeastern Alaska (3s, 4s, 5s of 1954 brood year)		Chum		31.5		1,795

ranging from 45 to 70% among the life-history types. The average number of circuli beyond the last annulus ranged from 0.7 to 1.9. In general, younger fish tended to have more new growth than older fish. The 4_2s had formed an average of 1.9 circuli whereas 5_2s averaged only 0.9 circulus after the annulus. The scale patterns of 5_3s and 6_3s were similar to the 4_2s and 5_2s, respectively: the 5_3s had an average of 1.4 circuli after the annulus while 6_3s had 0.7.

All scales in the samples taken in April 1965 showed new growth, having an average of 2.6–4.7 circuli after the annulus, from 1.9 to 2.8 more than the 1964 January samples (Fig. 3, Table I). In both series the younger fish tended to have more circuli after the annulus than did the older fish, and the 5_3 fish more than the 5_2 fish.

To explore the possibility that sockeye caught in different east-west regions of the Gulf might form their annulus and start new growth at different times, the samples were divided into three groups: those between 135°W and 143°W, those between 144°W and 151°W, and those between 152°W and 160°W. Between the two more westerly of the three geographical groups mentioned (Table I), the differences in number of "new" circuli were not consistent among the different life-history types, and all were statistically nonsignificant. The most easterly group contained only two life-history types and these in small numbers; however, in both cases they had the most new-growth circuli and the fewest fish lacking new circuli, of the three longitudinal divisions. The differences are not statistically significant but may be suggestive.

Our data suggest that the average time of annulus formation for sockeye present in the central part of the Gulf of Alaska in midwinter is sometime in January. The samples indicate a much earlier time of annulus formation than that suggested by Birman for Aleutian sockeye, i.e. in March.

Pink Salmon

Of 95 scales of first-year pink salmon taken from November 3 to 14, 1963, close to the British Columbia coast, mostly from Hecate Strait and Chatham Sound near the Skeena River estuary and from Dixon Entrance, 30% had a band of compactly spaced circuli (Fig. 4, 5) at the edge of their scales. For these scales there was an average of 18.8 circuli from the focus to the edge of the scale; the average number of compact circuli was three so that the beginning of the closely-spaced circuli was at about circulus 17, on the average. This indicates that formation of the annual ring for some fish had started in November or earlier.

It is most unlikely this band of compactly spaced circuli could be a "supplementary" check. Bilton and Ricker (1965) reported that on scales of marked pink salmon of known age returning to the Bella Coola River in central British Columbia the supplementary band of narrowly-spaced circuli, when it occurred, on the average began at about circulus 7 (as compared with 17, above) and ended at circulus 10 (the check). Thus, the supplementary check is evidently formed much earlier than November.

FIG. 2. The scale of a 4_2 sockeye salmon caught in January 1964, showing new growth (B) after the last annulus (A).

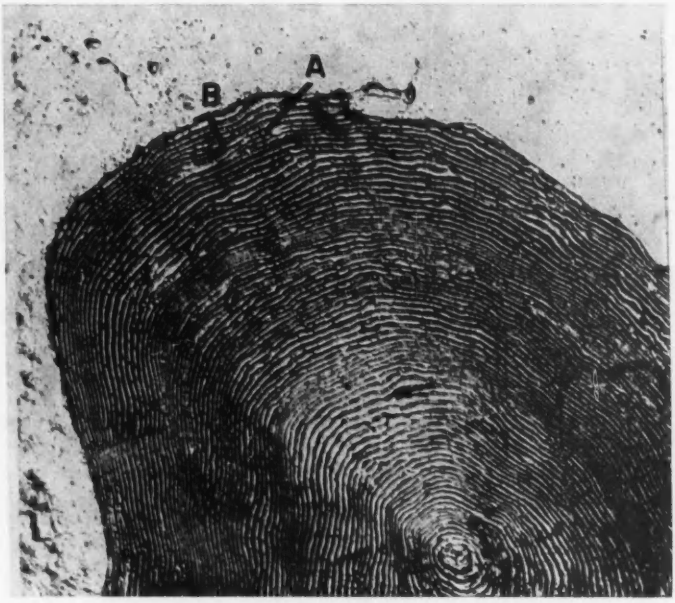

FIG. 3. The scale of a 5_2 sockeye salmon caught in April 1965, showing new growth (B) after the last annulus (A).

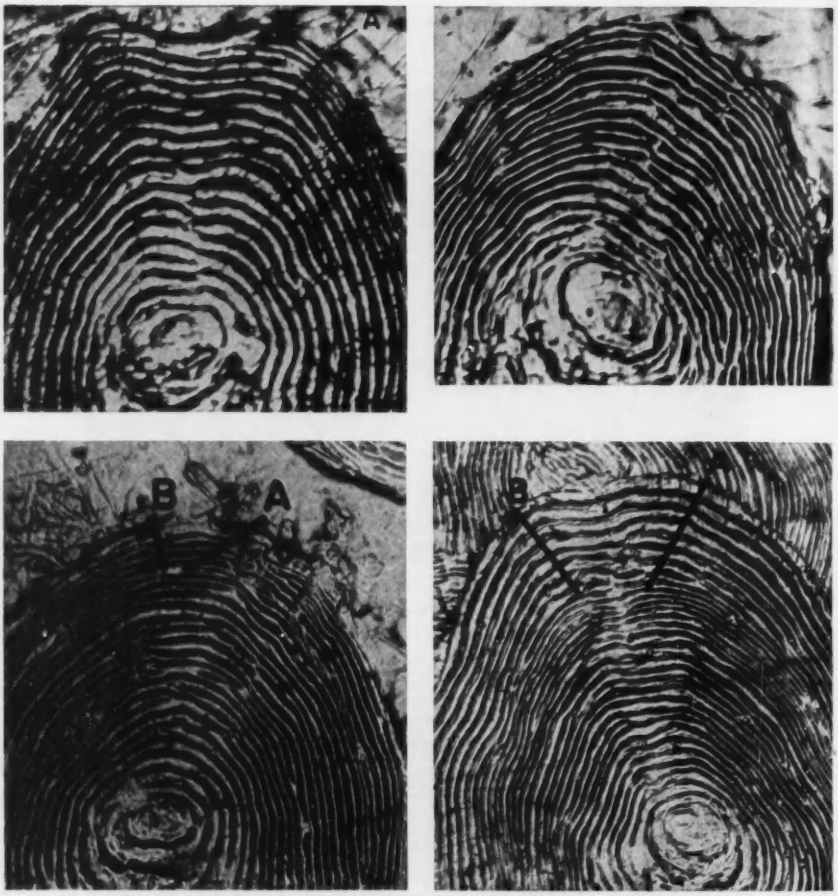

FIG. 4. *Top left.* The scale of a first-year pink salmon caught in November 1963, showing the annual ring (A) at the edge of the scale.

FIG. 5. *Top right.* The scale of a first-year pink salmon caught in November 1963, showing the absence of an annual ring at the edge of the scale.

FIG. 6. *Bottom left.* The scale of a second-year pink salmon caught in January 1964, showing new growth (B) after the annulus (A).

FIG. 7. *Bottom right.* The scale of a second-year pink salmon caught in April 1964, showing new growth (B) after the annulus (A).

FIG. 8. The scale of a first-year chum salmon caught in November 1963, showing the annual ring (A) at the edge of the scale.

FIG. 9. The scale of a first-year chum salmon caught in November 1963, showing the absence of an annual ring at the edge of the scale.

FIG. 10. The scale of a 4_1 chum salmon caught in January 1965, showing the annual ring (A) at the edge of the scale.

FIG. 11. The scale of a 3_1 chum salmon caught in April 1964, showing new growth (B) after the last annulus (A).

In the November samples the average number of circuli from the focus to the edge of the scale (18.8; range, 12–24) was considerably less than the average of 26.2 circuli (range, 20–36) from the focus to the annulus found on the scales of 300 mature British Columbia pink salmon taken in coastal fisheries the following summer.[3] Thus it appears that between early November 1963 and time of formation of the annulus an average of over seven more circuli would have been formed. Birman showed that, at the end of October and in November, pinks along the west coast of southern Sakhalin Island in the Sea of Okhotsk exhibited roughly the same number (average 17) as did the juvenile Hecate Strait pinks. However, none of the Sakhalin specimens had begun to form an annual ring.

Most of a sample of 23 scales of second-year pink salmon caught in the Gulf of Alaska from January 7 to February 7, 1964, had definite annuli; 78% showed new growth after the annulus, an average of 1.7 circuli for the entire sample (Fig. 6 and Table I).

New growth was present on the scales of all 97 pink salmon caught in the Gulf of Alaska during April 1964 (Fig. 7 and Table I). These had an average of 6.6 circuli, 4.9 circuli more than the January samples. At the average rate indicated—about 1.6 circuli per month—this suggests the average time of annulus formation was in late December, with the start of formation of the annual ring for some occurring in early November. This is earlier in the season than was suggested by Vedensky and Semko, who concluded that the annulus was formed later in the spring, but is at about the time of year suggested by Birman, i.e. prior to the middle of January.

Chum Salmon

Of the scales of 65 first-year chum salmon caught near the British Columbia coast in Hecate Strait, Chatham Sound, and Dixon Entrance from November 3 to 14, 1963, 29% had a band of compactly spaced circuli (Fig. 8, 9) at the edge. This suggested that formation of the annual ring for some had started in November or earlier. For the entire sample there was an average of 22.9 circuli (range, 16–32) from the focus to the edge of the scale. This was considerably less than the average of 31.5 circuli (range, 24–40) in the first year for 3-, 4-, and 5-year-old British Columbia chums (1795 in sample) of the 1954 brood year taken by the fishery in coastal waters as matures. Thus, it appears that between early November 1963 and time of formation of the annulus, an average of over eight circuli would have been formed. Subsequent examination of the scales of 14 age 3_1 and 4_1 chum salmon caught in the Gulf of

[3]The distance from the center of the focus to the tenth circulus for each of the scales from the juvenile pink salmon caught in Hecate Strait, Dixon Entrance, and Chatham Sound was measured. These values were compared with the same measurements made by Pearson (MS, 1965) for scales of pinks of known origin from British Columbia and Alaska. The average distance of 0.386 mm for the juveniles was identical with that of scales of pink salmon originating from the Bella Bella area in central British Columbia (which had an average distance of 0.386 mm for the first 10 circuli contrasted to 0.431 mm for pinks from Anan Bay in Southeast Alaska, 0.436 mm for pinks from Prince William Sound, 0.441 for pinks from Larson Bay in Kodiak). It is, therefore, likely that the juveniles had originated in British Columbia.

Alaska during January of 1964 and 1965 suggested that chum salmon form their annulus at a later date than either sockeye or pink salmon. All the scales examined had an annual ring (not necessarily complete) at the periphery of the scale (Fig. 10) but none had any new growth (Table I). However, all of a sample of 54 scales taken in the Gulf of Alaska during April 1964 had two or more circuli (range, 2–10) beyond the last annulus (Fig. 11 and Table I). Depending upon the age, the average number of circuli beyond the last annulus ranged from 4.4 to 5.3. Like sockeye, the younger fish (3_1s) had laid down more circuli than the older (4_1s) (3s had an average 5.3 circuli beyond the last annulus compared to 4.4 circuli for 4s). These results indicate that for some, the annual ring began to form as early as November, while the annulus was formed sometime in February or March. As outlined earlier, other scientists have placed the time of annulus formation for chums in the western North Pacific much later on, in April or May.

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A Simple Two-Dimensional Electrical Analog Model for Wind-Driven Circulation in the Gulf of St. Lawrence[1]

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ABSTRACT

A mathematical model of water circulation in an enclosed basin was examined, taking into account the effects of vertical friction, Coriolis force, and horizontal pressure gradients. Wind stress at the free surface was the only excitation force taken into account. The resulting vorticity equation was applied to the Gulf of St. Lawrence by using an electrical analog simulation technique. Solutions were obtained for two situations: one in which the circulation was vertically averaged and another which showed the circulation at several depths. Comparison of the results with broad features of the Gulf circulation derived from synoptic surveys suggested that the wind plays an important role in producing the long-term circulation. The model did not duplicate all of the features of the long-term circulation, but suggested several phenomena for further direct study.

INTRODUCTION

The difficulties involved in making comprehensive synoptic observations over large sea areas are enormous (see review by Webster, 1964). In the Gulf of St. Lawrence direct work of this nature has established the existence of certain features of the circulation. However, a completely clear picture of the *overall* circulation pattern of the Gulf has not yet emerged. The recent publication of Bumpus and Lauzier (1965) shows the general features of the circulation pattern at different seasons. One feature common to all of their data is the cyclonic nature of the circulation pattern. In recent years increasing use has been made of self-recording moored instruments and the amount and quality of current data collected has improved greatly (Farquharson 1958, personal communication, 1965).

An alternative method for obtaining information about the circulation process is to view the water masses as a mathematically defined hydrodynamic system set into motion by various driving forces. Models based on the hydrodynamic equations of motion can be constructed and the controlled effects of individual driving forces on them may be studied in isolation. Unfortunately, the complexity of the basic hydrodynamic equations makes it extremely difficult to cope with a rigorous mathematical model and for this reason simplifying assumptions must be made. Such simplifications will undoubtedly affect the results obtained and this should not be forgotten when interpreting these

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results. Nevertheless, such models can still be very valuable in ascertaining the effect of a given driving force in maintaining the circulation. In the Gulf of St. Lawrence, with the exception of the report by Jarlan (1961), very little attention has been given to this approach.

In making such a study of the dynamic circulation consideration must first be given to the driving forces involved. In a region such as the Gulf of St. Lawrence the most important of these are: (a) tidal forces; (b) changing atmospheric pressure at the free surface; (c) wind stress at the free surface; and (d) thermohaline and surface slope forces associated with density and pressure distributions established by agents other than (a), (b), and (c); these phenomena would include such things as evaporation, precipitation, river discharge, heating, and cooling. If interest is confined to average currents over extended periods, say 1 month or more, then it is usually assumed, to a first approximation, that phenomena (a) and (b) make no contribution.

The development of a model of thermohaline circulation is extremely difficult. However, a simplified model of the wind-driven circulation can be developed relatively easily. As an initial attempt at evaluating the effects of the various driving forces on the circulation the present study is thus limited to the wind-driven circulation only. A study of this nature is important in the Gulf because of the high wind speed and high cyclonic vorticity (Blackford and Tsang, MS, 1964) present in the average wind field over the Gulf.

SYMBOLS AND UNITS

x, y, z	[m]	Three axes of rectangular cartesian coordinate system: x axis directed eastward; y axis directed northward; z axis directed vertically upward.
u, v, w	[m sec^{-1}]	Velocity components along the three coordinate axes, respectively.
f	[sec^{-1}]	Coriolis parameter
β	[m^{-1} sec^{-1}]	Rate of change of Coriolis parameter with latitude.
P	[newtons m^{-2}]	Pressure
ρ	[kg m^{-3}]	Density
τ_x, τ_y	[newtons m^{-2}]	x and y components of stress inside fluid, respectively.
τ_{sx}, τ_{sy}	[newtons m^{-2}]	x and y components of surface stress, respectively.
g	[m sec^{-2}]	Acceleration due to gravity.
$\bar{u}, \bar{v}$	[m sec^{-1}]	Vertical averages of x and y velocity components, respectively.
ψ	[m^2 sec^{-1}]	Streamfunction for $\bar{u}$ and $\bar{v}$.

R	[kg m^{-2} sec^{-1}]	Bottom friction coefficient.
z_s, z_B	[m]	Coordinates of surface and bottom, respectively.
D	[m]	Defined as $(z_s - z_B)$
A_V	[kg m^{-1} sec^{-1}]	Vertical coefficient of eddy viscosity.
n	[ ]	Positive integer.
u_n, v_n	[m sec^{-1}]	Fourier coefficients in velocity expansion.
ψ_n	[m^2 sec^{-1}]	Streamfunction for u_n, v_n.
$\psi_{(z)}$	[m^2 sec^{-1}]	Streamfunction for u and v as a function of z.
h	[m]	Depth of no motion.
l	[m]	Horizontal grid spacing in two-dimensional network.
V	[volts]	Voltage in electrical analog.
I	[amperes]	Current in electrical analog.
R_x, R_y	[ohms]	Resistances in x and y directions of electrical analog, respectively.
$10^N K$	[volts m^{-2} sec] [m^2 ohm^{-1}]	Scale factor.

PHYSICAL AND MATHEMATICAL ASSUMPTIONS

The Vertically Averaged Model

The small size of the Gulf of St. Lawrence permits the use of a rectangular cartesian coordinate system, since the effects of sphericity are negligible. In the right-handed system used the x axis is positive eastward, the y axis is positive northward, and the z axis is positive upward. The origin is located at the mean free surface. A steady state is assumed and the nonlinear inertial terms are neglected. The frictional forces arising from horizontal shear are disregarded and in the vertical direction a hydrostatic balance is assumed. With these assumptions and using the notation given in the table of symbols, the hydrodynamic equations of motion at a particular point can be written as:

$$f\rho v + \frac{\partial \tau_x}{\partial z} = \frac{\partial P}{\partial x} \qquad \ldots\ldots (1)$$

$$-f\rho u + \frac{\partial \tau_y}{\partial z} = \frac{\partial P}{\partial y} \qquad \ldots\ldots (2)$$

$$-g\rho = \frac{\partial P}{\partial z} \qquad \ldots\ldots (3)$$

and the continuity equation, assuming that density ρ is constant, gives:

$$\frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} + \frac{\partial w}{\partial z} = 0 \qquad \ldots\ldots (4)$$

A further simplification is obtained by eliminating the details of the z direction and this is accomplished by vertical integration of the above equations. Thus, integrating equation 1 vertically from the bottom z_B to the surface z_s gives:

$$f\rho\int_{z_B}^{z_s} v\,dz + \tau_{sx} - \tau_{Bx} = \int_{z_B}^{z_s} \frac{\partial P}{\partial x}\,dz$$

where τ_{sx} and τ_{Bx} are the stresses at the surface and bottom, respectively. Also, since the density is assumed constant[2] the horizontal pressure gradient is independent of depth and can be taken outside the integral. This equation may then be rewritten as follows:

$$f\rho\bar{v}\,(z_s - z_B) + \tau_{sx} - R\bar{u} = \frac{\partial P}{\partial x}\,(z_s - z_B) \qquad \ldots\ldots (5)$$

where $\bar{u}$ and $\bar{v}$ are the averages of the x and y velocity components over a vertical column, and it is assumed that the frictional force on the bottom is given by $\tau_{Bx} = R\bar{u}$ (Stommel, 1948). Similarly, equations 2 and 4 may be integrated vertically to give:

$$-f\rho\bar{u}\,(z_s - z_B) + \tau_{sy} - R\bar{v} = \frac{\partial P}{\partial y}\,(z_s - z_B) \qquad \ldots\ldots (6)$$

$$\frac{\partial}{\partial x}\left[\bar{u}\,(z_s - z_B)\right] + \frac{\partial}{\partial y}\left[\bar{v}\,(z_s - z_B)\right] = 0 \qquad \ldots\ldots (7)$$

where it has been assumed in equation 7 that there is no loss of water through the surface or bottom of the sea under consideration. Furthermore, if $(z_s - z_B)$ is assumed independent of x and y, equation 7 becomes:

$$\frac{\partial \bar{u}}{\partial x} + \frac{\partial \bar{v}}{\partial y} = 0 \qquad \ldots\ldots (8)$$

The arbitrary assumption that $(z_s - z_B)$ is constant has a direct physical interpretation. It means that the topography of the sea bottom is exactly the same as that of the sea surface. Since the maximum value of $|z_s|$ is of the order of 1 m, and that of $|z_B| \sim 200$ m, the departure from a model with a level bottom is very slight. It should also be noted that this assumption has

[2]In Appendix II a model with variable density is considered. It is found that a similar vorticity equation is obtained by making an appropriate assumption concerning the pressure gradient at the bottom.

no significant effect on the pressure gradient. In the paper by Stommel (1948) the equivalent of equation 8 in the present report is an approximate equation. Here equation 8 is exact and the assumption made to make it so can be seen physically to have a negligible effect. See also the report by Garner (1962, p. 41–42).

The pressure gradient terms may now be eliminated by cross-differentiating equations 5 and 6 and subtracting the resulting equations, that is:

$$\frac{\partial f}{\partial y}\rho\bar{v}\,(z_s - z_B) + f\rho\,(z_s - z_B)\frac{\partial \bar{v}}{\partial y} + \frac{\partial \tau_{sx}}{\partial y} - R\frac{\partial \bar{u}}{\partial y} = \frac{\partial^2 P}{\partial x \partial y}(z_s - z_B) \quad \ldots\ldots (9)$$

and

$$-f\rho\,(z_s - z_B)\frac{\partial \bar{u}}{\partial x} + \frac{\partial \tau_{sy}}{\partial x} - R\frac{\partial \bar{v}}{\partial x} = \frac{\partial^2 P}{\partial x \partial y}(z_s - z_B) \quad \ldots\ldots (10)$$

Subtracting equation 10 from equation 9 and substituting from equation 8 then gives:

$$\frac{\partial f}{\partial y}\rho\bar{v}\,(z_s - z_B) + \frac{\partial \tau_{sy}}{\partial y} - \frac{\partial \tau_{sy}}{\partial x} - R\frac{\partial \bar{u}}{\partial y} + R\frac{\partial \bar{v}}{\partial x} = 0 \quad \ldots\ldots (11)$$

From equation 8 a streamfunction ψ may be introduced such that flow at any point is parallel to lines of constant ψ.

Thus:

$$\bar{u} = \frac{\partial \psi}{\partial y} \text{ and } \bar{v} = -\frac{\partial \psi}{\partial x} \quad \ldots\ldots (12)$$

Substituting equation 12 into equation 11 then gives:

$$R\left(\frac{\partial^2 \psi}{\partial x^2} + \frac{\partial^2 \psi}{\partial y^2}\right) + \beta\rho D\frac{\partial \psi}{\partial x} = -\left(\frac{\partial \tau_{sy}}{\partial x} - \frac{\partial \tau_{sx}}{\partial y}\right) \quad \ldots\ldots (13)$$

where $\frac{\partial f}{\partial y} = \beta$, a constant. (For further discussion of the β-plane approximation see Fofonoff, 1962a). Equation 13 is a *vorticity* equation and represents a balance among the sum of *frictional* and *planetary* vorticity on the left side of the equation and the *anemographic* (wind stress) vorticity on the right.

The basic problem from this point on is that of finding solutions to equation 13 from a knowledge of the x, y distribution of surface wind stress. Of course, the solutions must satisfy certain boundary conditions at the solid shore line. The boundary condition is very simple mathematically and is derived physically from the fact that no flow can occur in a direction normal to the solid boundary. In other words the flow must be parallel to the solid boundaries and therefore ψ must be constant on the solid boundaries.

THE VERTICALLY STRUCTURED MODEL

The system of equations 1–4 can also be reduced by a method similar to that used by Hassan (1958, 1964). The resulting vorticity equation is similar to equation 13 but the function corresponding to ψ is a different entity. This method permits an examination of the vertical structure of the circulation pattern. The full derivation is lengthy and only the initial assumptions and final results are given here. The interested reader is referred to the papers of Hassan (1958, 1964), or to Blackford (MS, 1965) for a discussion of this particular case.

Referring to equations 1–4 Hassan introduces the additional assumptions that:

(1) the vertical component of velocity, w, is everywhere equal to zero, and

(2) the frictional stresses resulting from vertical shear may be simulated by use of a constant eddy viscosity coefficient, i.e.

$$\tau_x = A_V \frac{\partial u}{\partial z} \text{ and } \tau_y = A_V \frac{\partial v}{\partial z}$$

The vorticity equation resulting from this method is

$$\left(\frac{\partial^2 \psi_n}{\partial x^2} + \frac{\partial^2 \psi_n}{\partial y^2}\right) + \frac{4\rho\beta h^2}{(2n-1)^2 \pi^2 A_V} \frac{\partial \psi_n}{\partial x} = \frac{8h}{(2n-1)^2 \pi^2 A_V} \left(\frac{\partial \tau_{sx}}{\partial y} - \frac{\partial \tau_{sy}}{\partial x}\right) \quad \ldots\ldots (14)$$

To find the set of functions ψ_n, equation 14 must be solved for each value of n. Theoretically n goes to infinity but for practical purposes the series must be terminated at a finite value. Having obtained in this way an adequate set of ψn's the streamfunction for u and v at any depth may be calculated by using the following equation:

$$\psi(z) = \sum_{n=1}^{K} \psi_n \cos \frac{(2n-1)\,\pi z}{2h} \quad \ldots\ldots (15)$$

where $\psi(z)$ is a function of z as well as of x and y. The velocity components u and v are then determined from $\psi(z)$ by the usual relation:

$$u = \frac{\partial \psi(z)}{\partial y} \text{ and } v = -\frac{\partial \psi(z)}{\partial x}.$$

This method gives a three dimensional picture of the velocity field in a sea with constant depth of no motion. However, it does this at the expense of neglecting the vertical velocity. As pointed out by Stommel (1957) and Robinson (1964), this is a very serious restriction, particularly in a water body of oceanic dimensions.

Equation 14 is similar in form to equation 13. In fact they differ only in the constants and unknown function. Thus, any method devised to solve

equation 13 can also be used to solve equation 14. The electrical analog technique was used to apply both of these equations to the Gulf of St. Lawrence.

THE ELECTRICAL ANALOG MODEL

As pointed out in the previous section, the solution to equation 13 must be obtained in a water body of very irregular geometry, and under the action of a known but irregular wind-stress pattern. Equation 13 can be solved analytically for a water body of regular shape acted upon by a wind-stress pattern having a convenient analytic representation. In fact, both Stommel (1948) and Hassan (1958) have done this for an ocean of rectangular shape. However, in the Gulf of St. Lawrence the irregular geometric shape and presence of islands have an important effect on the circulation and therefore must be taken into account. Thus, an electrical analog simulation technique is preferred to the analytic method of solution.

Electrical analog simulation is based on the mathematical similarity between the equation relating streamfunction to wind-stress curl (i.e. equation 13) in the hydrodynamic model and the equation relating voltage to current in an electrical impedance network. The objective is thus to find an electrical impedance network whose governing equation is similar in form to equation 13. This impedance network is then constructed to have the same geometric shape as the Gulf. Boundary conditions and wind stress driving forces of the hydrodynamic system are appropriately scaled and applied to the electrical analog system. Voltage is proportional to streamfunction and electrical input current is proportional to wind-stress curl. The voltage distribution at the nodes of the electrical network may be measured directly. The streamfunction distribution in the hydrodynamic system is obtained from this voltage distribution by applying the appropriate scaling factor. Velocity components are obtained by making use of equation 12.

To make the transition from the hydrodynamic system, equation 13 may be rewritten in the more concise form:

$$\left(\frac{\partial^2\psi}{\partial x^2}+\frac{\partial^2\psi}{\partial y^2}\right)+C_1\frac{\partial\psi}{\partial x}=C_2\left(\frac{\partial\tau_{sx}}{\partial y}-\frac{\partial\tau_{sy}}{\partial x}\right) \qquad \ldots\ldots (16)$$

where

$$C_1=\frac{\beta\rho D}{R} \text{ and } C_2=\frac{1}{R} \text{ in the Stommel case;}$$

OR $C_1=\dfrac{4\beta\rho h^2}{(2n-1)^2\,\pi^2\,A_V}$ and $C_2=\dfrac{8h}{(2n-1)^2\,\pi^2\,A_V}$ in the Hassan case, where ψ is replaced by ψ_n. Since any physically constructed electrical network has a finite grid spacing, equation 16 must be written in finite-difference form. By using the values of ψ at the nodes of the finite grid network of Fig. 1 and

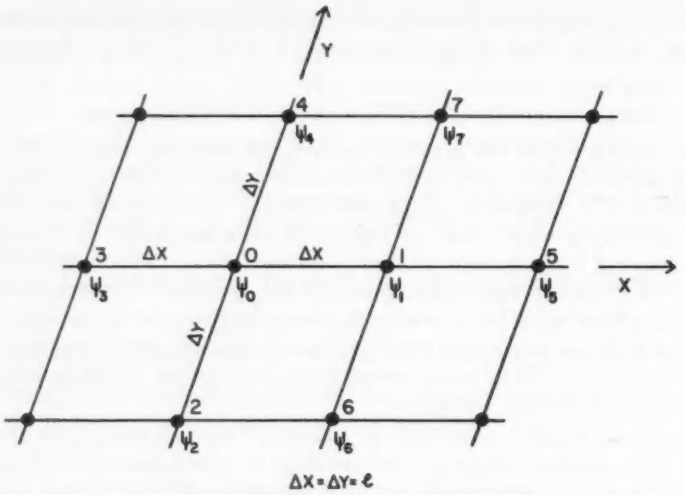

FIG. 1. Portion of two dimensional space; used to derive equation 17.

remembering the basic definition of a derivative, the finite-difference form of equation 16 taken about node 0 is:

$$\frac{1}{l}\left[\frac{\psi_1-\psi_0}{l}-\frac{\psi_0-\psi_3}{l}\right]+\frac{1}{l}\left[\frac{\psi_4-\psi_0}{l}-\frac{\psi_0-\psi_2}{l}\right]+C_1\frac{(\psi_1-\psi_0)+(\psi_0-\psi_3)}{2l}=$$

$$C_2\left[\frac{\tau_{sx4}-\tau_{sx2}}{2l}-\frac{\tau_{sy1}-\tau_{sy3}}{2l}\right] \quad \ldots\ldots (17)$$

or after rearranging:

$$\left[\left(\frac{1}{l^2}+\frac{C_1}{2l}\right)\left(\psi_1-\psi_0\right)+\left(\frac{1}{l^2}-\frac{C_1}{2l}\right)\left(\psi_3-\psi_0\right)+\frac{\psi_4-\psi_0}{l^2}+\frac{\psi_2-\psi_0}{l^2}\right]10^N K_0=$$

$$10^N K_0\left[\frac{C_2}{2l}\left(\tau_{sx4}-\tau_{sx2}-\tau_{sy1}+\tau_{sy3}\right)\right] \quad \ldots\ldots (18)$$

where $10^N K_0$ is an arbitrary scaling factor.

The equation relating voltage to current in the resistance network of Fig. 2 is obtained by applying the law of conservation of charge at node 0. Thus:

$$\frac{V_1-V_0}{R_{xm}}+\frac{V_3-V_0}{R_{x(m-1)}}+\frac{V_4-V_0}{R_{ym}}+\frac{V_2-V_0}{R_{ym}}=I_0 \quad \ldots\ldots (19)$$

where I_0 is defined as the electrical current *out of* node 0.

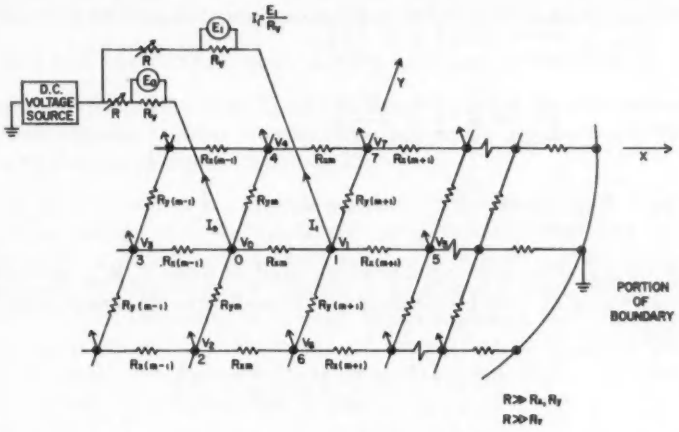

FIG. 2. Portion of resistance network in the electrical analog (arrows indicate node currents); used to derive equation 19.

Examining equations 18 and 19 shows that there is a term-by-term correspondence between them, allowing a formal comparison to be made. Thus:

$$V = 10^N \psi$$

$$I_0 = 10^N K_0 \frac{C_2}{2l}\left[\tau_{sx4} - \tau_{sx2} - \tau_{sy1} + \tau_{sy3}\right]$$

$$\frac{1}{R_{xm}} = K_0\left(\frac{1}{l^2} + \frac{C_1}{2l}\right)$$

$$\frac{1}{R_{x(m-1)}} = K_0\left(\frac{1}{l^2} - \frac{C_1}{2l}\right) \qquad \ldots\ldots (20)$$

and

$$\frac{1}{R_{ym}} = \frac{K_0}{l^2}$$

Now if the above procedures are repeated at node 1 in Fig. 2 instead of node 0, then the following results are obtained:

$$V = 10^N \psi$$

$$I_1 = 10^N K_1 \frac{C_2}{2l}\left[\tau_{sx7} - \tau_{sx6} - \tau_{sy5} + \tau_{sy0}\right]$$

$$\frac{1}{R_{x(m+1)}} = K_1\left(\frac{1}{l^2} + \frac{C_1}{2l}\right)$$

$$\frac{1}{R_{xm}} = K_1\left(\frac{1}{l^2} - \frac{C_1}{2l}\right) \qquad \ldots\ldots (21)$$

$$\frac{1}{R_{y(m+1)}} = \frac{K_1}{l^2}$$

where K_1 is the arbitrary constant being used at node 1. R_{xm} occurs in both equations 20 and 21, and for consistency it must have the same value in each case. Thus:

$$\frac{K_1}{K_0} = \left(\frac{1}{l^2} + \frac{C_1}{2l}\right) \div \left(\frac{1}{l^2} - \frac{C_1}{2l}\right) \qquad \ldots\ldots (22)$$

It can be seen from equations 20 and 21 that:

$$\frac{R_{xm}}{R_{x(m+1)}} = \frac{K_1}{K_0}$$

$$\frac{R_{ym}}{R_{y(m+1)}} = \frac{K_1}{K_0} \qquad \ldots\ldots (23)$$

Equation 23 requires that both the x and y resistance values must increase by the ratio K_1/K_0 for each step in the negative x direction. Thus, in the electrical model the resistance values must be highest at the western boundary and decrease in the eastward direction. The absolute values of resistance are determined by the choice of the arbitrary constant K_0.

The choice of K_0 in the present model is dictated by practical considerations. The largest resistance values at the western boundary must be of a size which can be obtained experimentally. Having chosen this starting point the progressively smaller resistors to the east are determined by repeated applications of equation 23. As shown in equation 22 the ratio K_1/K_0 is dependent on the grid size l and on C_1. Recalling that C_1 is directly proportional to the coefficient of the planetary vorticity term in equation 13 it can be seen that K_1/K_0 is equal to unity in a region where this term is negligible (i.e. $C_1 = 0$). Stommel (1948) has shown that there is no westward intensification of the streamfunction in this case. In the corresponding electrical model all of the resistances are of equal value (equation 23) and thus there is no intensification of voltage in the western regions. On the other hand, when C_1 is larger $\frac{K_1}{K_0}$ becomes greater than unity[3] and there is a westward intensification of streamfunction and voltage in the hydrodynamic and electrical models, respectively.

[3]Peculiarities occur as C_1 increases. However, these will not be discussed here and the interested reader is referred to the works of Karplus (1958) and Karplus and Soroka (1959).

It can also be seen from equations 20 and 21 that the scaling factor relating wind stress curl to electrical input current increases by the ratio $\frac{K_1}{K_0}$ for each step in the positive x direction. Thus, the electrical input currents are weighted and become stronger toward the east. This relation is inverse to the change in resistance values as shown in equation 23.

To feed electrical current into the resistance network of Fig. 2, a d-c voltage source (E) is connected to each node through a variable resistance (R). These variable resistors are adjusted to give the proper current values. The currents are measured by metering the voltage drop across a small resistor (R_V) in series with R. The resistance of R must be much greater than R_x, R_y, so that the input currents will be determined by the setting of R alone. Otherwise, interactions would occur between the currents at each node making it extremely tedious to obtain the proper input currents.

Figure 3 shows a resistance network used in the present model. The electrical currents are fed into the nodes of this network by the array of variable

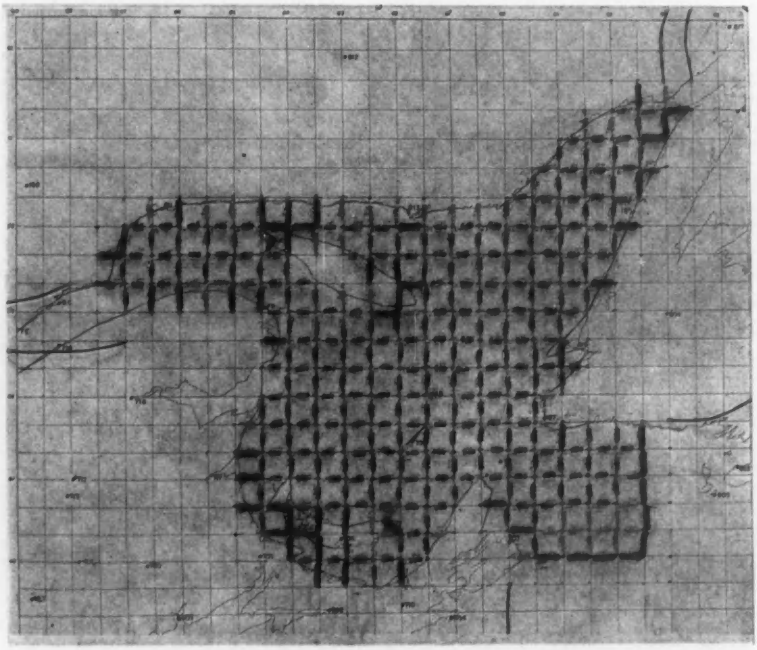

FIG. 3. Resistance network for electrical analog model of Gulf of St. Lawrence.

resistors shown in Fig. 4. The constants of equation 13 are chosen to have the following values:

$$\beta = 1.5 \times 10^{-11}\ \mathrm{m^{-1}\ sec^{-1}}$$

$$\rho = 10^{3}\ \mathrm{kg\ m^{-3}}$$

$$D = 2 \times 10^{2}\ \mathrm{m} \qquad \ldots\ldots (24)$$

$$R = 2 \times 10^{-1}\ \mathrm{kg\ m^{2}\ sec^{-1}}$$

$$l = 3.7 \times 10^{4}\ \mathrm{m}$$

Thus, from equation 16, $C_1 = 1.5 \times 10^{-5}\ \mathrm{m^{-1}}$
and $C_2 = 5\ \mathrm{kg^{-1}\ m^{2}\ sec.}$
With these values of l and C_1 the value of K_1/K_0 (equation 22) in the electrical model becomes:

$$K_1/K_0 = 1.77.$$

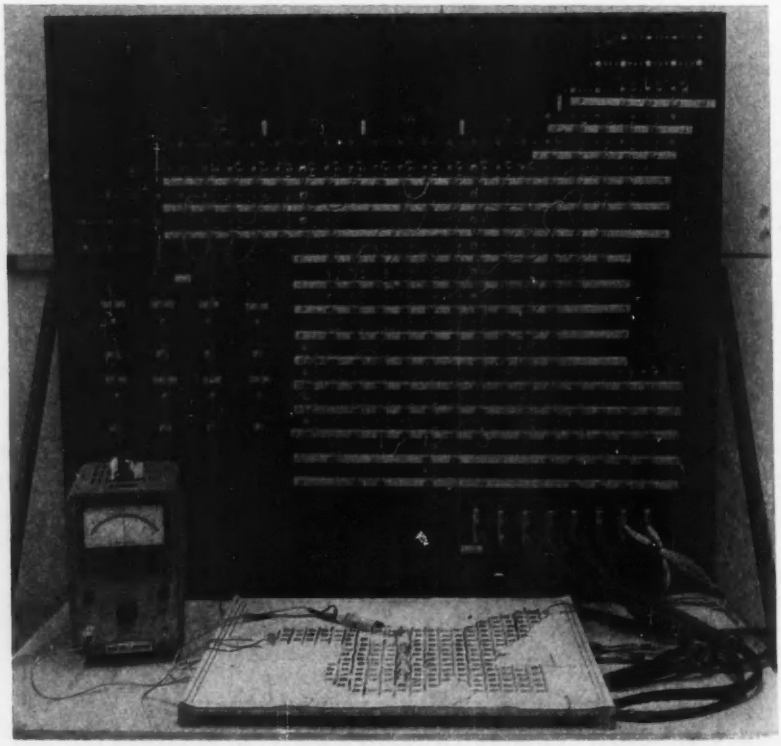

FIG. 4. Array of variable resistors used to introduce electrical currents into the grid points of the resistance network of Fig. 3.

The value of the constant K_0 at the western boundary is then arbitrarily chosen to be 5×10^2 ohm^{-1} m^2. This leads to resistance values at the western boundary of the order of 10^7 ohms. At the eastern boundary the resistance values are reduced by repeated application of equation 23 to the order of 10 ohms. The value of the exponent in the scaling factor is chosen to be minus five, i.e. $N = -5$.

The values of D and R in equation 24 were chosen quite arbitrarily. The given value of D is a rough average depth for the gulf and the value of R is the same as that used by Stommel (1948). This value of R does have some support from qualitative arguments based on dimensional analysis (see Appendix I). These values appear to represent limits in both cases, an upper limit on D and a lower limit on R. Thus, the ratio D/R is at the maximum which could be expected for this model in the Gulf of St. Lawrence. Even at this the ratio of K_1/K_0 is already rather small. If the values of D and R are altered so that the ratio D/R is reduced by an order of magnitude then the effect of the planetary vorticity term is completely negligible. For example, this would occur if one chose D = 20 m, which is a rough average thermocline depth in summer. Thus, as a general statement it can be said that this term is probably relatively unimportant in the gulf. However, its effect is readily noticeable with the values of D and R given in equation 24, as will be seen in the next section. It should also be noted that increasing the value of R reduces the effect of wind stress, i.e. increasing R decreases the flow velocities obtained.

The network of Fig. 3 can also be used to solve the equation of Hassan, i.e. equation 14, for $n = 1$. The same value of C_1 is obtained if h and A_V are chosen as follows:

$$h = 200 \text{ m}$$

$$A_V = 16 \text{ kg m}^{-1} \text{ sec}^{-1}.$$

This again probably represents an upper limit on the coefficient C_1. The value of C_2 is 10 kg^{-1} m^2 sec in this case. For $n > 1$ the coefficient C_1 is reduced by at least an order of magnitude and the planetary vorticity term can be neglected. In this case a resistance network with equal values of resistance throughout, except near the boundaries, is used.

It was mentioned earlier that the boundary condition on ψ is of the form ψ = constant along the solid shoreline. In the resistance network this corresponds to voltage V being constant along the solid shoreline. This is accomplished extremely easily by constructing the shoreline of a low-resistance wire. At the open boundaries such as Cabot Strait, the streamfunction is not constant. In fact the flow through the opening is proportional to the change in streamfunction from one side to the other. A similar argument is also true for voltage at the open boundaries in the resistance network.

Results obtained with this model of the Gulf of St. Lawrence are given in the following section. Solutions to both the Stommel and Hassan equations are shown. Only one pattern of wind-stress curl is used, however, it is typical

of the long-term average (Blackford and Tsang, MS, 1964). The effect of applying various boundary conditions and also the effect of the planetary vorticity term are studied.

RESULTS

Figure 5 shows the locations of weather stations whose data are used in constructing the atmospheric pressure charts. The rectangular grid network is the same as that used in the electrical analog resistance network. Figure 6 is an atmospheric pressure chart showing pressure distribution for the period December–April and is based on an average of observations from 1940 to 1949. The geostrophic wind is parallel to the isobars so that it can be seen that there is a cyclonic type of flow in the wind pattern. Figure 7 shows the values of wind-stress curl at the nodes of the grid network of Fig. 5. These wind-stress curls were calculated from the pressure chart of Fig. 6 by a method similar to that used in the report mentioned above (see also Fofonoff, 1962b). The input currents for the electrical analog model were calculated from the wind-stress curls of Fig. 7 by a simple multiplication factor (see equation 20).

The data obtained from the model are presented in the form of contoured plots of streamfunction values, the resulting lines being streamlines. For example, in Fig. 8 the flow is parallel to the streamlines at any point and the direction is indicated by the arrows on the streamlines. The numbers indicate streamfunction values on the contours. Streamfunction values are also shown

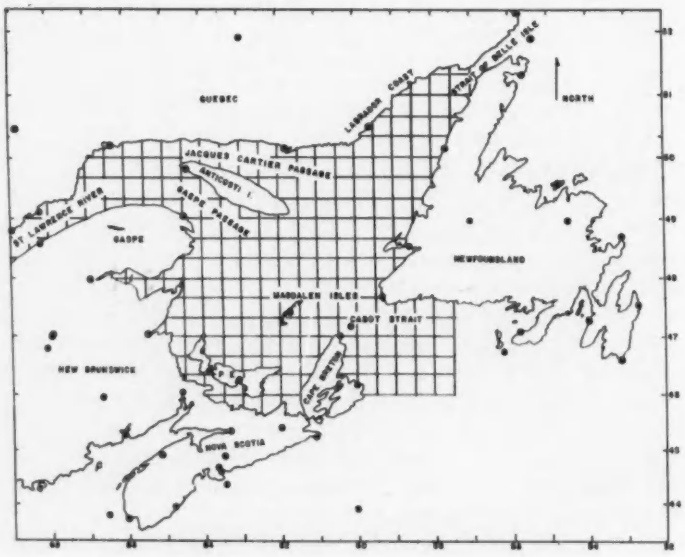

FIG. 5. Map of the Gulf of St. Lawrence and surrounding area (black dots indicate Weather Stations).

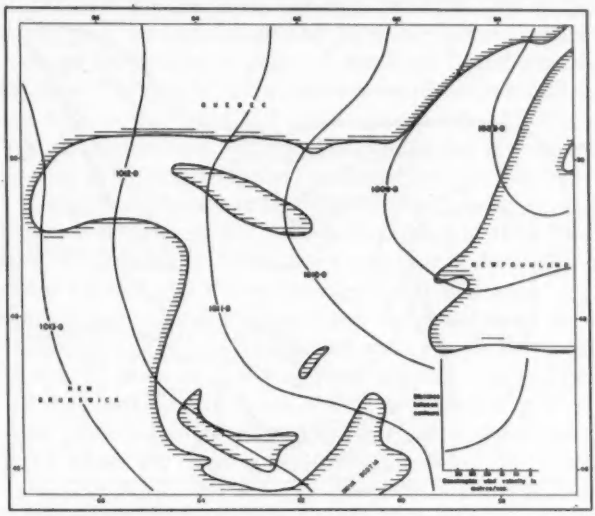

FIG. 6. Atmospheric pressure chart (millibars) for the period December–April based on average of data from 1940 to 1949.

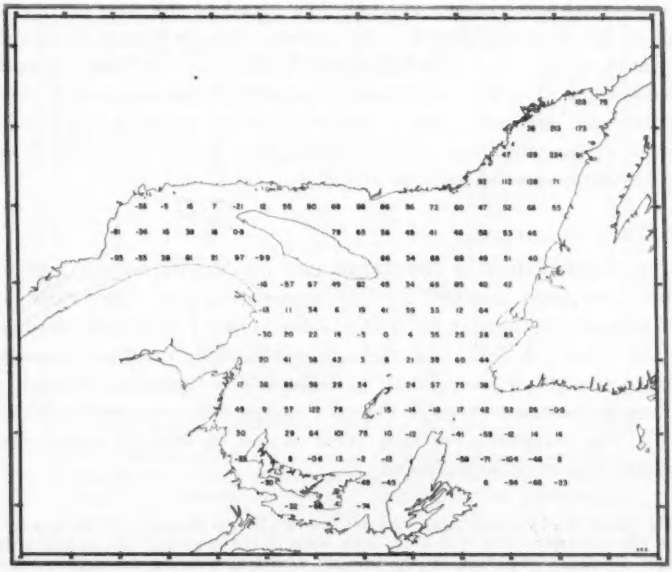

FIG. 7. Wind stress curls at nodes of electrical analog resistance network (values in 10^{-8} newton/$_{m^3}$).

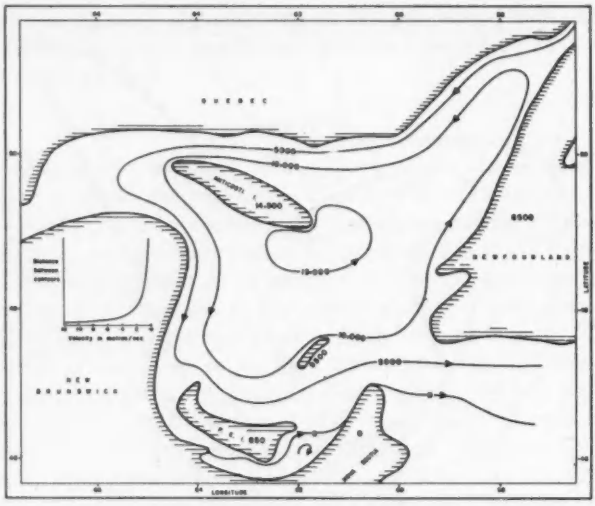

FIG. 8. Horizontal flow pattern of vertically averaged velocities. The planetary vorticity effect is included. The Strait of Belle Isle and Cabot Strait are open, and also Gaspé and Jacques Cartier passages (see Fig. 5).

on the mainland[4] and various islands. The speed of the flow is inversely proportional to the distance between the contour lines and may be calculated by making use of the graph at the left of the figure. Two features are common to all of these figures: firstly, the general anticlockwise nature of the overall flow pattern and, secondly, the existence of closed gyres in the region to the southeast of Anticosti Island. It should be noted that there are no closed gyres in the corresponding wind pattern of Fig. 6.

STOMMEL-TYPE SOLUTIONS

In Fig. 8 the values of streamfunction on Newfoundland and Anticosti Island have not been imposed by any external means. They arise from the effect of the input currents at the nodes of the network. This results in an inflow through the Strait of Belle Isle and a corresponding outflow through Cabot Strait. All of the inflow from Strait of Belle Isle is funnelled through Jacques Cartier Passage and out through Gaspé Passage. This feature is enhanced by the effect of the planetary vorticity term as can be seen by comparison with Fig. 12 where this term is neglected.

[4]In Fig. 8 and the following figures no net flow is allowed through the St. Lawrence River opening, i.e. the streamfunction is zero on both sides of the opening. This is equivalent to neglecting river discharge. However, in the present model of wind-driven circulation including this would not make much change in the streamfunction pattern obtained, except near the river opening. This does not mean that I consider the river discharge to have negligible effect on the circulation pattern of the Gulf. It is only in the present model that it is being neglected.

In Fig. 9 the streamfunction on Anticosti Island is arbitrarily set at zero, preventing a net flow through Gaspé and Jacques Cartier passages. The flow is deflected around the southeast point of Anticosti Island and higher speeds are produced there. The speeds in the region west of Anticosti Island are quite low (less than 0.05 m/sec), and the small arrows indicate only the general directions. The streamfunction value on Newfoundland is still determined by the input currents and has not changed appreciably from Fig. 8.

In Fig. 10 the streamfunction on Newfoundland is set equal to zero and that on Anticosti is determined by the input currents at the nodes of the grid network. The flow is reduced considerably to the east of Anticosti but the flows through Jacques Cartier and Gaspé passages are still strong. In Fig. 11 the streamfunction on Anticosti is also zero and the effect is to deflect the flow around the southeast end of Anticosti Island as in Fig. 9. However, the speeds are smaller in Fig. 11 since no flow is allowed through Strait of Belle Isle.

In Fig. 12 the input conditions are similar to those of Fig. 8, so that differences in the flow pattern are due entirely to the effect of the planetary vorticity term. In Fig. 12, where this term is neglected, the inflow through the Strait of Belle Isle[5] is much stronger than in Fig. 8. However, a much smaller proportion of this flow is funnelled through Jacques Cartier Passage and the current through

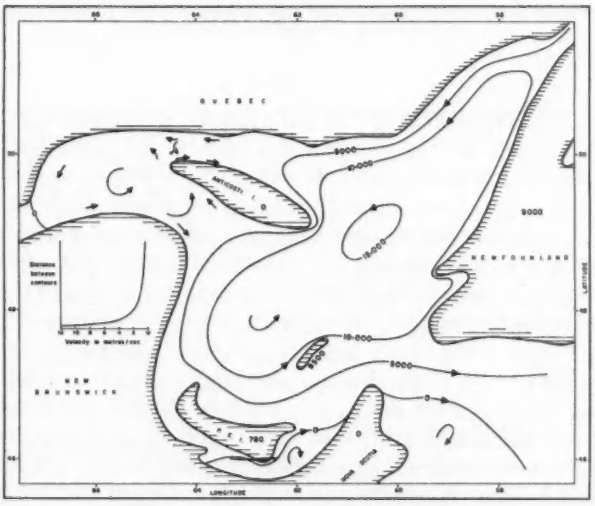

FIG. 9. Horizontal flow pattern of vertically averaged velocities. The planetary vorticity effect is included. The Strait of Belle Isle and Cabot Strait are open. Gaspé and Jacques Cartier passages are closed.

[5]The inflow through Strait of Belle Isle in Fig. 8, 9, 12, and 13 is probably on the high side. In the actual gulf depths in this strait are less than the 200 m assumed in the model so that the effects of friction would be higher than depicted in the model.

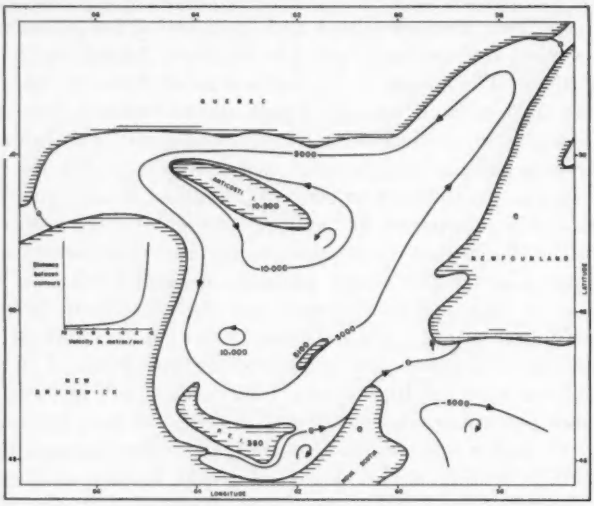

FIG. 10. Horizontal flow pattern of vertically averaged velocities. Planetary vorticity effect is included. The Strait of Belle Isle and Cabot Strait are closed. Gaspé and Jacques Cartier passages are open.

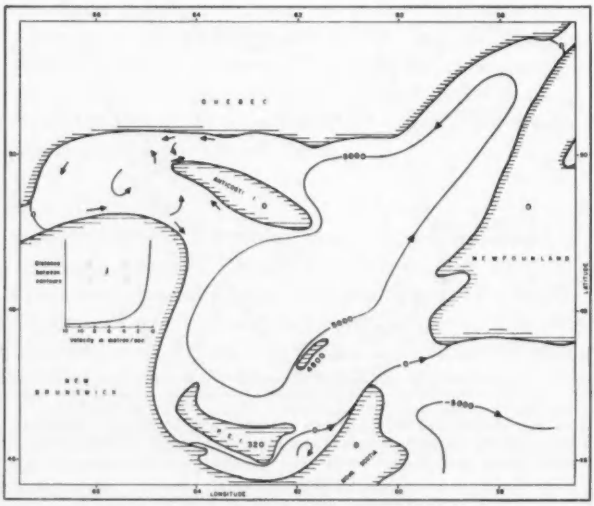

FIG. 11. Horizontal flow pattern of vertically averaged velocities. Planetary vorticity effect is included. The Strait of Belle Isle and Cabot Strait are closed. Gaspé and Jacques Cartier passages are closed.

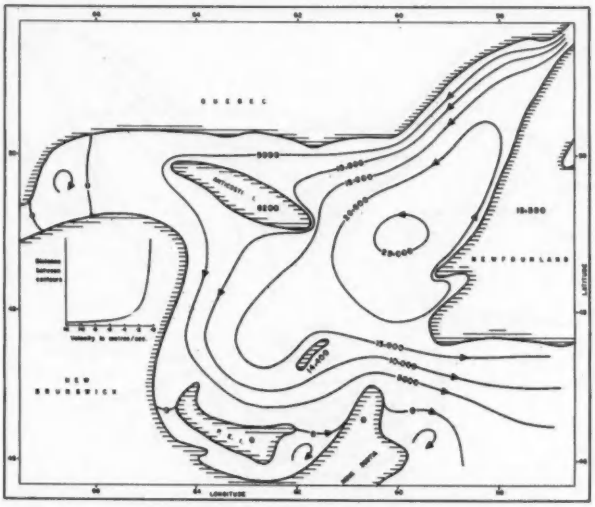

FIG. 12. Horizontal flow pattern of vertically averaged velocities. Planetary vorticity effect is neglected. Compare with Fig. 8 to see the effect of the planetary vorticity term.

this passage is considerably weaker than in Fig. 8. The current strength east of Anticosti has increased considerably and the gyre in this area has shifted eastward. The eastward flow across the Magdalen Shallows has also increased.

In Fig. 13 the streamfunction on Anticosti is set equal to zero, otherwise the input conditions are similar to those in Fig. 12. The flow through the Strait of Belle Isle has not changed noticeably and the imposed lack of flow through Jacques Cartier Passage has caused an increase in current strength to the east of Anticosti. A small gyre is present west of the Magdalen Islands, as in Fig. 9.

In Fig. 14 no net flow is allowed through the Strait of Belle Isle or Cabot Strait. The value of the streamfunction on Anticosti is determined by the input currents. Comparing Fig. 14 with Fig. 10 shows the effects of neglecting the planetary vorticity term. In Fig. 14 the flow through Jacques Cartier Passage is less than in Fig. 10. To the east of Anticosti the flows are much stronger in Fig. 14. In particular the northeast flow along the west coast of Newfoundland is much stronger in Fig. 14. Also the eastward flow across the Magdalen Shallows is enhanced by neglecting the planetary vorticity term. In Fig. 15 Anticosti Island is also set at zero streamfunction, as in Fig. 11. The flows are much stronger in the latter figure where the planetary vorticity term is neglected.

Generally speaking, the planetary vorticity term strengthens the current through Jacques Cartier and Gaspé Passages. At the same time currents in other parts of the Gulf are weakened by its presence.

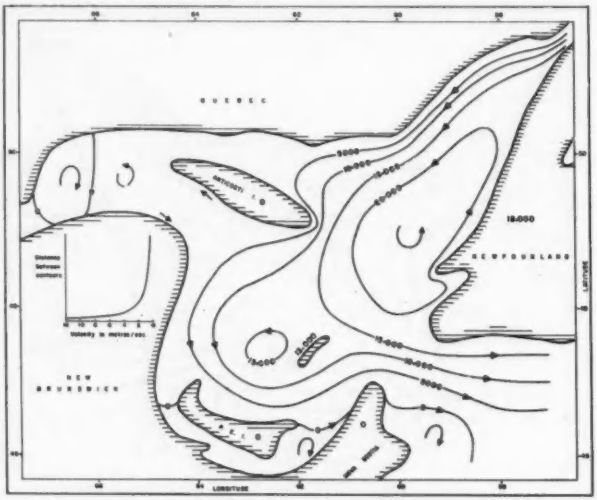

FIG. 13. Horizontal flow pattern of vertically averaged velocities. Planetary vorticity effect is neglected. Compare with Fig. 9 to see the effect of the planetary vorticity term.

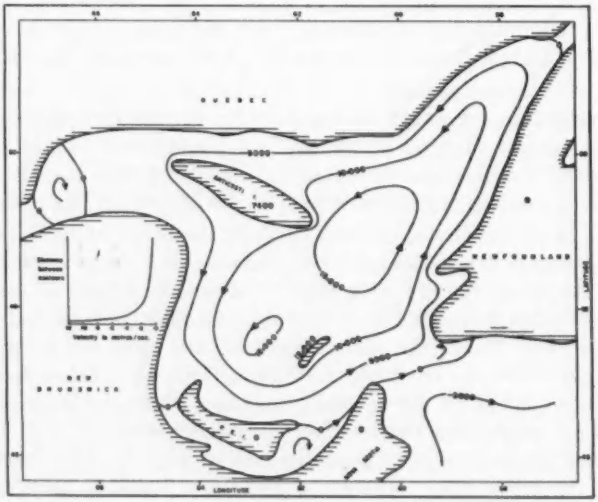

FIG. 14. Horizontal flow pattern of vertically averaged velocities. Planetary vorticity effect is neglected. Compare with Fig. 10 to see the effect of the planetary vorticity term.

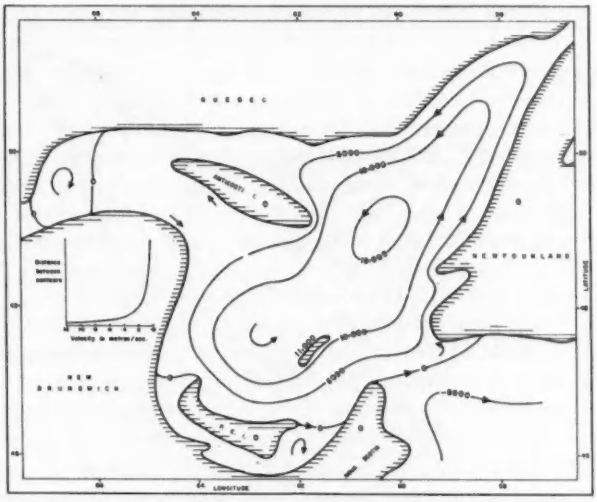

FIG. 15. Horizontal flow pattern of vertically averaged velocities. Planetary vorticity effect is neglected. Compare with Fig. 11 to see the effect of the planetary vorticity term.

HASSAN-TYPE SOLUTIONS

Equation 14 was solved for $n = (1,10)$ using the wind stress data of Fig. 7. Using equation 15, values of streamfunction at depths of 0, 20, 40, 100, and 150 m were then calculated. The general flow patterns were much the same as those obtained with the Stommel-type solutions. The surface speeds were higher than the vertically averaged velocities. There were no drastic changes in the circulation pattern with increasing depth, simply a gradual decrease of velocity to zero at the assumed 200-m depth of no motion. Figures 16 and 17 show the circulation at the surface and 100 m, respectively.

DISCUSSION

The main features of the flow patterns produced by the model are: (1) An inflow through the Strait of Belle Isle with an equal outflow through Cabot Strait; (2) An inflow through Jacques Cartier Passage with an equal outflow through Gaspé Passage; (3) A fairly strong southerly flow curving clockwise around the east end of Anticosti Island; (4) A southerly flow along the New Brunswick shore and an easterly flow across the Magdalen Shallows; (5) A northeasterly flow along the west coast of Newfoundland; (6) The existence of closed gyres to the southeast of Anticosti Island and on the northern part of the Magdalen Shallows.

The majority of these features are in very good agreement with the results of synoptic observations as shown by Bumpus and Lauzier (1965), also Lauzier

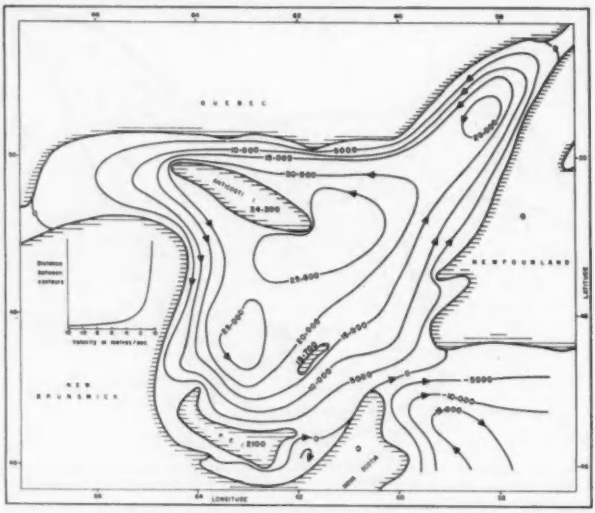

FIG. 16. Surface flow pattern (Hassan equation). Planetary vorticity effect is included. The Strait of Belle Isle and Cabot Strait are open, and also Gaspé and Jacques Cartier passages.

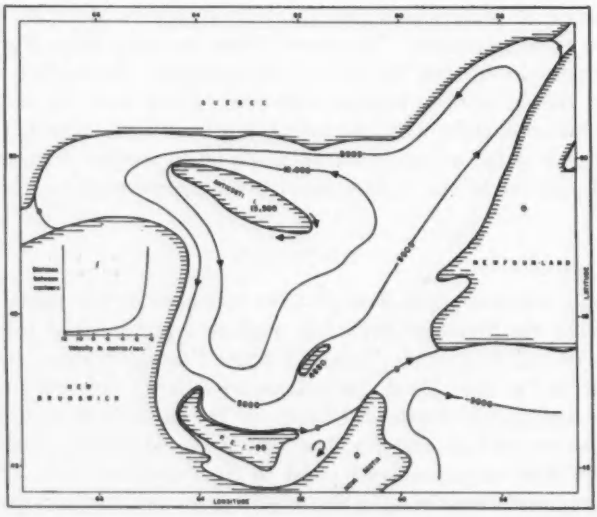

FIG. 17. Flow pattern at 100 m (Hassan equation). Planetary vorticity effect is included. The Strait of Belle Isle and Cabot Strait are open, and also Gaspé and Jacques Cartier passages.

(1965). Furthermore, the speeds produced by the model are of the right order of magnitude.

In Gaspé Passage synoptic observations (Farquharson, personal communication) have established the existence of a strong outward current (the Gaspé current) on the Gaspé coast side of the Passage. Also, there appears to be a weaker inflow on the Anticosti Island side. The mechanism of this current system is not understood. The outflow through Gaspé Passage in the wind-driven model (see Fig. 8) is strong on the Gaspé coast side of the passage but there is no inflow on the Anticosti side. Also, this outflow is associated with an equal inflow through Jacques Cartier. This suggests that simultaneous measurements of flows in Gaspé and Jacques Cartier passages would help to determine if the average wind plays a role in producing the Gaspé current. A better (but more costly) experiment would be to make a comprehensive survey over a three-dimensional grid in the region north of latitude 40° and west of longitude 61°. This would allow horizontal streamlines to be plotted so that a more conclusive evaluation of the model results could be obtained.

Also, in Cabot Strait synoptic observations (MacGregor, 1956) indicate an inflow on the Newfoundland side and an outflow (not necessarily equal) on the Cape Breton side. This feature is not particularly prominent in the results of the present model. However, it must be remembered that this model considers only one driving force, i.e. the wind. Undoubtedly, tidal forces and estuarine mechanisms also have an effect, especially in places like Cabot Strait and Gaspé Passage.

The effects of inertia and horizontal friction are absent in the model, so that there is no mechanism for producing eddy motions on the downstream side of islands and land protrusions, etc. The effects of bottom topography are also neglected. This undoubtedly will have an effect on the flow pattern, especially in regions where bottom slopes are large.

CONCLUSIONS

Despite the many factors neglected in the model it has provided a very definite suggestion that the wind is an important factor in producing the long-term circulation. Furthermore, the electrical analog technique has proved to be practical for oceanographic problems of this nature. The basic simplicity of the model and the ease with which irregular boundaries and wind stress can be accommodated make the technique particularly attractive.

ACKNOWLEDGMENTS

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APPENDIX I

By applying the methods of dimensional analysis to equation 13 one can obtain an idea of the value of the friction coefficient R to be used in the Gulf. Thus, rewriting equation 13 gives:

$$R\left(\frac{\partial^2\psi}{\partial x^2}+\frac{\partial^2\psi}{\partial y^2}\right)+\beta\rho D\,\frac{\partial\psi}{\partial x}=\frac{\partial\tau_{sx}}{\partial y}-\frac{\partial\tau_{sy}}{\partial x}$$

Now this equation must be written in nondimensional form (compare Langhaar, 1951, and Fofonoff, 1962). To facilitate this operation the following notation is introduced:

$$\psi=\bar{\psi}\,\psi_0 \qquad R=\bar{R}\,R_0$$

$$x=\bar{x}\,L \qquad \beta=\bar{\beta}\,\beta_0 \qquad \tau_{sx}=\bar{\tau}_{sx}\,\tau_0$$

$$y=\bar{y}\,L \qquad D=\bar{D}\,H \qquad \tau_{sy}=\bar{\tau}_{sy}\,\tau_0$$

The bar quantities are nondimensional variables and ψ_0, L, H, R_0, β_0, and τ_0 are characteristic values of the variables, respectively. These characteristic values depend on the size and location of the area under study. Substituting these expressions into equation 13 and rearranging gives the following nondimensional equation:

$$\bar{R}\left(\frac{\partial^2\bar{\psi}}{\partial\bar{x}^2}+\frac{\partial^2\bar{\psi}}{\partial\bar{y}^2}\right)+\frac{\beta_0\rho_0 HL}{R_0}\left(\bar{\beta}\,\bar{\rho}\,\bar{D}\,\frac{\partial\bar{\psi}}{\partial\bar{y}}\right)=\frac{\tau_0 L}{R_0\psi_0}\left(\frac{\partial\bar{\tau}_{sx}}{\partial\bar{y}}-\frac{\partial\bar{\tau}_{sy}}{\partial\bar{x}}\right)$$

In many parts of the ocean, particularly where the meridional velocity is zero, the vorticity tendency of the windstress term must be balanced by the vorticity tendency of the frictional term (Stommel, 1951). This means that these two terms must be of the same order of magnitude. Thus, the coefficient of the wind-stress term in the nondimensional equation must be of unit magnitude (Fofonoff, 1962). Thus

$$\frac{\tau_0 L}{R_0\psi_0}\sim 1$$

or

$$R_0\sim\tau_0\Big/\frac{\psi_0}{L}.$$

Now ψ_0/L is the order of magnitude of the characteristic velocity U_0. In Stommel's (1948) ocean $\tau_0\sim 0.1$ newtons/^{2} and $U_0\sim 1m$/sec. Thus, $R_0\sim 0.1$ kg m^{-2} sec.$^{-1}$ Stommel picked a value of R_0 to get velocity values which agreed

with those observed in the real ocean. His value of 0.2 kg m^{-2} sec^{-1} agrees well with that derived above on the basis of dimensional arguments.

With the value of R_0 thus obtained the coefficient multiplying the planetary vorticity term in the nondimensional equation may be calculated. For Stommel's ocean this gives:

$$\frac{\beta_0 \rho_0 HL}{R_0} \sim 50.$$

Thus, the planetary vorticity term is extremely important in regions where the meridional velocity is not zero.

As far as the Gulf of St. Lawrence is concerned, the same argument applies to the wind stress term in relation to the frictional term, thus:

$$R_0 \sim \tau_0 \Big/ \frac{\psi_0}{L}.$$

Now the wind-stress values (Blackford and Tsang, MS, 1964) over the Gulf appear to be somewhat larger than the values used by Stommel (1948) and also the observed characteristic velocities are somewhat smaller. Thus, the value of R_0 might be expected to be somewhat larger than that used in Stommel's case. In any case, it appears that the value for R_0 used in the present report represents a lower limit. The coefficient multiplying the planetary vorticity term is reduced by about two orders of magnitude from its value in Stommel's report. This is apparent since the linear dimensions of the Gulf are about one-tenth those of the ocean. Thus, the importance of the planetary vorticity term is greatly reduced from what it is in the ocean. Intuitively, one would expect this to be the case.

APPENDIX II

In this appendix the vorticity equation is developed by a method paralleling that of the first part of the section on Physical and Mathematical Assumptions. The only difference here is that the density is not assumed constant. This permits the possibility of much greater changes in pressure gradients and velocities with depth below the surface. It can no longer be assumed that the pressure gradient is independent of depth. It can be verified that the resulting vorticity equation is similar to equation 13, provided that certain assumptions are made about the pressure gradients at the bottom.

Rewriting equations 1, 2, 3, and 4 gives:

$$f\rho v + \frac{\partial \tau_x}{\partial z} = \frac{\partial P}{\partial x} \qquad \ldots\ldots (1a)$$

$$-f\rho u + \frac{\partial \tau_y}{\partial z} = \frac{\partial P}{\partial y} \qquad \ldots\ldots (2a)$$

$$-g\rho = \frac{\partial P}{\partial z} \qquad \ldots\ldots (3a)$$

$$\frac{\partial}{\partial x}(\rho u) + \frac{\partial}{\partial y}(\rho v) + \frac{\partial}{\partial z}(\rho w) = 0 \qquad \ldots\ldots (4a)$$

Integrating equation 1a vertically as before gives:

$$f\int_{z_B}^{z_s} \rho v dz + \tau_{sx} - \tau_{Bx} = \int_{z_B}^{z_s} \frac{\partial P}{\partial x}\, dz \qquad \ldots\ldots (5a)$$

Similarly, from equations 2a and 4a,

$$-f\int_{z_a}^{z_s} \rho u dz + \tau_{sy} - \tau_{By} = \int_{z_B}^{z_s} \frac{\partial P}{\partial y}\, dz \qquad \ldots\ldots (6a)$$

and

$$\frac{\partial}{\partial x}(\overline{\rho u}) + \frac{\partial}{\partial y}(\overline{\rho v}) = 0 \qquad \ldots\ldots (7a)$$

Differentiating equation 5a with respect to y gives:

$$\frac{\partial f}{\partial y}\int_{z_B}^{z_s} \rho v dz + f\frac{\partial}{\partial y}\int_{z_B}^{z_s} \rho v dz + \frac{\partial \tau_{sx}}{\partial y} - \frac{\partial \tau_{Bx}}{\partial y} = \frac{\partial}{\partial y}\int_{z_B}^{z_s} \frac{\partial P}{\partial x}\, dz$$

$$\text{or } \frac{\partial f}{\partial y} D\overline{v} + fD\frac{\partial}{\partial y}\overline{v} + \frac{\partial \tau_{sx}}{\partial y} - \frac{\partial \tau_{Bx}}{\partial y} = \int_{z_B}^{z_s} \frac{\partial^2 P}{\partial x \partial y} dz + \frac{\partial z_s}{\partial y}\left(\frac{\partial P}{\partial x}\right)_s - \frac{\partial z_B}{\partial y}\left(\frac{\partial P}{\partial x}\right)_B \qquad \ldots\ldots (8a)$$

since $\frac{\partial P}{\partial x}$ is no longer independent of z. Similarly, differentiating equation 6a with respect to x gives:

$$-fD\frac{\partial \overline{\rho u}}{\partial x} + \frac{\partial \tau_{sy}}{\partial x} - \frac{\partial \tau_{By}}{\partial x} = \int_{z_B}^{z_s} \frac{\partial^2 P}{\partial x \partial y}\, dz + \frac{\partial z_s}{\partial x}\left(\frac{\partial P}{\partial y}\right)_s - \frac{\partial z_B}{\partial x}\left(\frac{\partial P}{\partial y}\right)_B \quad \ldots\ldots (9a)$$

Subtracting equation 9a from equation 8a and substituting from equation 7a gives:

$$D\frac{\partial f}{\partial y}\rho\bar{v} + \left(\frac{\partial \tau_{sx}}{\partial y} - \frac{\partial \tau_{sy}}{\partial x}\right) + \left(\frac{\partial \tau_{By}}{\partial x} - \frac{\partial \tau_{Bx}}{\partial y}\right) = \left[\frac{\partial z_s}{\partial y}\left(\frac{\partial P}{\partial x}\right)_s - \frac{\partial z_s}{\partial x}\left(\frac{\partial P}{\partial y}\right)_s\right] + \left[\frac{\partial z_B}{\partial x}\left(\frac{\partial P}{\partial y}\right)_B - \frac{\partial z_B}{\partial y}\left(\frac{\partial P}{\partial x}\right)_B\right] \quad \ldots\ldots (10a)$$

The first term in square brackets on the right-hand side of equation 10a is identically zero, so that it gives no contribution. Now, as before, let $\tau_{Bx} = R^1\overline{\rho u}$ and $\tau_{By} = R^1\overline{\rho v}$. At the same time, define a streamfunction ψ^1 such that $\frac{\partial \psi^1}{\partial x} = -\overline{\rho v}$ and $\frac{\partial \psi^1}{\partial y} = \overline{\rho u}$.

Substituting these expressions into equation 10a gives:

$$R^1\left(\frac{\partial^2\psi^1}{\partial x^2} + \frac{\partial^2\psi^1}{\partial y^2}\right) + D\frac{\partial f}{\partial y}\frac{\partial \psi^1}{\partial x} = \frac{\partial \tau_{sx}}{\partial y} - \frac{\partial \tau_{sy}}{\partial x} + \left[\frac{\partial z_B}{\partial x}\left(\frac{\partial P}{\partial y}\right)_B - \frac{\partial z_B}{\partial y}\left(\frac{\partial P}{\partial x}\right)_B\right] \quad \ldots\ldots (11a)$$

The expression in square brackets on the right-hand side of equation 11a is the only term which distinguishes it from equation 13. This term will be identically zero if:

(1) z_B is independent of x and y

(2) pressure gradients are zero at the bottom

(3) lines of constant bottom pressure are parallel to bottom contour lines. This means that geostrophic velocity at the bottom is parallel to bottom contour lines.

In the present model condition 1 is nearly true since variations in z_B are the same as variations in z_s, and thus are small. If density variations are allowed, it is quite likely that condition 2 is nearly satisfied as well. Thus, the term in square brackets will be small here. On the other hand, in a model which takes bottom topography into account, this term would likely be important in regions where the bottom slopes are large.

Blood Volumes of Three Salmonids[1,2]

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ABSTRACT

Two species of salmon (*Oncorhynchus kisutch*, *O. nerka*) and steelhead and rainbow trout (*Salmo gairdneri*) were injected with Evans blue dye. Typical excretion slopes and a minimum mixing time of 60 min were determined by sampling blood through a dorsal aorta cannula for up to 5 hr after injection. Extrapolation of the excretion slope to zero time provided an estimate of the dye dilution which, with hematocrit measurements, gave blood volume estimates ranging from 5.4 ± 0.8 to 6.9 ± 1.8 ml/100 g.

A new method of using Evans blue was tested in which dye was injected into one fish and its plasma reinjected into other fish. Blood volume estimates for *O. kisutch* were 4.5 ± 1.5 by this method as compared with 6.2 ± 2.0 ml/100 g for Evans blue used in the usual manner in the same individuals.

Blood volumes estimated from plasma samples taken 20, 25, and 30 min after injection while the fish were maintained out of water were 35% less than after the same fish were returned to water and a second estimate made from additional samples taken 1, 2, and 3 hr after the original dye injection.

INTRODUCTION

The estimation of blood volumes involving the injection of substances into the blood and measuring their subsequent dilution has a long and complex history, with both physiologic and phylogenetic significance. The widely accepted conclusions are that teleost fish have the smallest blood volumes, 1.5–3% of body weight, of any of the vertebrates, the remainder having blood volumes in the range from 5 to 10% of body weight (Prosser and Brown, 1961, p. 388–389), and that the evolutionary trend in fish is toward smaller blood volumes (Thorson, 1961). Representative data and methods are shown in Table I. In mammals, 30 min is usually considered to be a satisfactory allowance for complete mixing of the injected material, and most studies on fish have used this assumption.

With the development of a blood-sampling technique which did not require restraint, anesthesia, or other disturbance of the fish's normal activities during experimentation (Smith and Bell, 1964), it became possible to study mixing times in a detailed fashion. When adequate mixing time is allowed, the extrapolation back to the time of dye injection to correct for the rate of dye excretion yields a dilution and hence a value for blood volume that is considered

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[2]Work carried out at the Biological Station, Nanaimo, B.C., for the Fisheries Research Board of Canada, and also supported in part by grant, A-1731, from the National Research Council of Canada.
[3]Present address: Fisheries Research Institute, University of Washington, Seattle, Wash.

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TABLE I. Representative blood volumes for teleost fish.

Scientific name	Blood volume (*ml/100 g*)	Method	Reference
Salmo gairdneri	3.5±0.9	Evans blue	Conte et al., 1963
Salmo gairdneri	3.3±0.9	I^{131} albumen	Conte et al., 1963
Salmo gairdneri	2.8±1.0	Cr^{51} RBC	Conte et al., 1963
Gadus morhua	2.4	Fluorescein	Ronald et al., 1964
Gadus morhua	1.9	Exsanguination	Ronald et al., 1964
Carassius carassius	2.5–3.0	Hb washout	Korzhuyev and Nikolskaya, 1951
Solea vulgaris	1.8–2.4	Hb washout	Korzhuyev and Nikolskaya, 1951
Belone vulgaris	4.0–5.9	Hb washout	Korzhuyev and Nikolskaya, 1951
Ophiodon elongatus	2.8	Evans blue	Martin, 1955
Several teleosts	1.8–3.8	Evans blue	Thorson, 1961

more accurate than values so far reported for salmonid fish. These blood volumes are sufficiently higher to demand a revision of the accepted values for this group of fish.

MATERIALS AND METHODS

The fish in these studies were from several stocks available at the Nanaimo Biological Station during the summers of 1963, 1964, and 1965. Smaller coho salmon (*Oncorhynchus kisutch*) (Table II, test 1) and steelhead trout (*Salmo gairdneri*) (Table II, test 7) were cultured in sea water at the Station and were about 2 years old when their blood volumes were estimated. Rainbow trout (*S. gairdneri*) (Table II, test 8) were obtained from a local trout farm and held in freshwater tanks. Sockeye salmon (*O. nerka*) (Table II, test 6) were trapped as adult upstream migrants in the Stamp River, near Port Alberni, B.C., and transported to the Station in oxygenated, chilled tanks where they were held in large freshwater tanks with weekly doses of sea water to control fungus infection. Larger coho (*O. kisutch*) (Table II, tests 2–5) were caught as immature adults in the Strait of Georgia near Nanaimo by a commercial troller and transported in running sea water to large seawater tanks at the Station, where they were held for several weeks to allow recovery from hook injuries. All experiments were carried out at seasonal environmental temperatures for the species concerned: seawater species, 8–12 C, and freshwater species, 11–16 C.

Handling methods were basically the same for all fish. After inserting a dorsal aorta cannula (Smith and Bell, 1964) and allowing at least 2 hr recovery from the M.S. 222 anesthesia, a measured amount of dye or tagged material was injected through the cannula and washed in with an equal amount of

TABLE II. Blood volumes of three salmonids by several methods.

Fish	Test No.	Weight (g)	No. of fish	Hematocrit (% *RBC*)	Environment	Plasma (*ml/100 g*)	Blood (*ml/100 g*)	Method
Coho salmon								
(*Oncorhynchus kisutch*)	1	537±108[a]	7	21±8.1[a]	Sea water	4.8±1.3[a]	6.2±1.6[a]	Evans blue
	2	1049±173	10	30±8.3	Sea water	4.3±0.7	6.1±0.9	Evans blue
	3a	949±23	3	33±7.5	Sea water	3.0±0.5	4.6±0.3	Evans blue[b]
	3b	949±23	3	33±7.5	Sea water	4.7±0.3	7.2±0.1	Evans blue[c]
	4a	926±112	7	25±5.4	Sea water	4.6±1.4	6.2±2.0	Evans blue
	4b	930±104	8	20±7.3	Sea water	3.4±1.0	4.5±1.5[d]	Reinjected Evans blue
	5	1012±120	6	–	Sea water	–	2.3±0.4	Exsanguination
Sockeye salmon								
(*O. nerka*)	6	1814±266	8	26±3.8	Fresh water	4.0±0.6	5.4±0.8	Evans blue
Salmo gairdneri Steelhead trout	7	553±127	13	21±7.8	Sea water	5.6±1.8	6.9±1.8	Evans blue
Rainbow trout	8	548±49	4	–	Fresh water	–	2.4±0.4	Exsanguination

[a]Mean and standard error.
[b]Estimates made while the fish were under M.S. 222 anesthesia on an operating table (belly up, gills irrigated with oxygenated sea water) from blood samples taken at 20, 25, and 30 min after injection of dye.
[c]Estimates made on the same individuals as in 3a, but after they had been placed in aquaria and allowed to recover from the anesthesia. Blood samples were taken at 1, 2, and 3 hr after the first dye injection without an additional dye injection.
[d]Values obtained by use of reinjected Evans blue into the same individual fish as for test 4a, with random selection as to whether Evans blue or reinjected Evans blue was used first. The reinjected Evans blue consisted of plasma from donor coho salmon which had received large doses of Evans blue 5–6 hr before centrifuging and reinjection. The second injection was given 48 hr after the first.

heparinized saline. Samples of 0.5 ml of blood were withdrawn periodically, beginning 1 hr after dye injection (see discussion of mixing time), and replaced with 1% NaCl or Cortland saline (Wolf, 1963). Hematocrits were determined before and after dye injections with a multiple-head hematocrit centrifuge giving about 13,000 *g*. From the plasma thus obtained, 0.2 ml was diluted to 3.0 ml and its optical density read in a Beckman DU spectrophotometer. The optical densities for several samples from each fish were plotted on semi-log paper and the excretion slope extrapolated to zero time to provide an estimate of the original dye dilution (Fig. 1).

Slight variations on traditional methods of using Evans blue were necessary. Evans blue (T-1824) dye stock solution was 1 mg dye/ml water and had an OD of 4.70 (measured as a 10-fold dilution). The quantities injected were

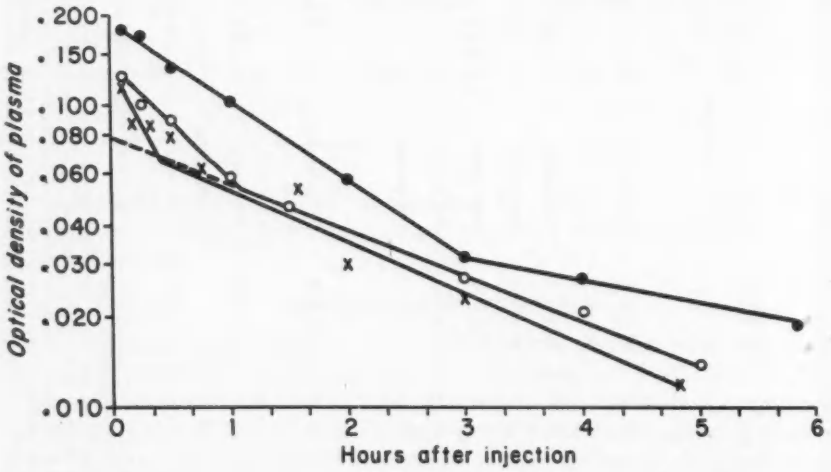

FIG. 1. Mixing times and excretion rates of Evans blue dye in salmon. Illustrated are an unusually long mixing time (●—●—●), typical mixing time (○—○—○), and an unusually short mixing time (×—×—×). Dashed line shows typical extrapolation.

0.8–1.5 ml, depending on the size of the fish. The OD of the dye in the plasma was read at 600 mμ rather than the usual 620 mμ because of a slight shift in color when diluted with 0.1% NaCl. The latter was necessary to prevent occasional clouding of the plasma samples. When injecting dye for a second volume estimate in an individual fish, plasma was used as a blank in the spectrophotometer so that any residual dye from the previous determination was eliminated.

Since it was desirable to confirm the volume estimates made by dye dilution with those made by other methods, P^{32} and I^{131} labelling of red blood cells and plasma proteins were attempted, using the technique of Conte et al. (1963), as well as fluorescein-labelling of plasma proteins after the method

of Ronald et al. (1964). Neither method provided a label which did not leach away during dialysis; and when the labelled material was injected into fish, dilutions occurred which indicated blood volumes in excess of 20% of body weight. Similar unsuccessful attempts were made to label plasma proteins with Evans blue *in vitro* and remove unbound dye by dialysis.

It was then decided to try *in vivo* dialysis. Sockeye weighing 1.5–2 kg tolerated injections of up to 15 ml of stock Evans blue solution (1 mg/ml), especially if injected in 5-ml aliquots at 30-min intervals. After 5–6 hr, as much blood as possible was removed from the fish and centrifuged to obtain the tagged plasma. Week-long dialysis of the labelled plasma showed no leakage of Evans blue into the dialysis bath. If the color of the plasma was so pale as to require injecting more than 2–3 ml for a blood volume estimate, the plasma was concentrated by air dialysis. In no case was any mortality or behavioral response to reinjected plasma noted, so it is believed that there was no allergic or immune response involved.

For exsanguination, blood was withdrawn from the dorsal aorta cannula with a syringe, but no attempt was made to empty the heart and other large blood spaces as did Ronald et al. (1964).

Additional methods are detailed in footnotes to Table II.

RESULTS

A typical mixing time for Evans blue dye is contrasted with an unusually short and an unusually long mixing time in Fig. 1. All show two slopes, one for mixing plus excretion and a later one for excretion. For most fish, the intersection between the two slopes occurred about 1 hr after injection. Two exceptional fish were observed, one in which there were three distinct decreases in slope and one in which there were no changes in slope. After these preliminary experiments, later blood volume estimates were made from hourly blood samples giving 3–5 points along the excretion slope for extrapolation, the earlier samples being omitted to conserve blood. The reinjected Evans blue appeared to mix and be excreted at rates similar to ordinary Evans blue.

The blood volume estimates, as well as being larger than commonly accepted for salmonids, indicated similarity of blood volumes at different sizes (test 1–2), decreased blood volume while out of water (test 3a), and a lesser volume estimated by the reinjected Evans blue method (test 4b). Exsanguination was intended not to give a realistic estimate of blood volume, but to indicate the quantity of readily available blood (test 5 and 8 — see Discussion).

DISCUSSION

As far as is known, no one has previously taken serial samples to examine the mixing of substances injected into the blood stream of fish. Martin (1955) sampled up to 90 min after injection and Ronald et al. (1964) sampled after 10 and 20 min and again after 1 and 2 weeks, but not periodically in either case.

Most other investigators sampled after 30 min following the practice adopted for mammals. The present data clearly indicate variable mixing times with 60 min as a practical minimum.

Further, mixing is probably not normal unless the fish is undisturbed in an adequate environment — not anesthetized, restricted, out of water, or hypoxic. All such conditions can depress heart rate in salmonids (Randall et al., 1965). In the case of blood volumes measured while the fish is out of water, one might suspect pooling of blood in the lowermost parts of the fish or collapse of vessels in the uppermost parts of the fish, but no critical test of this hypothesis was possible. The reduced blood volume estimated in fish on the operating table out of water (Table II, test 3a) was probably due to a combination of depressed circulation and incomplete mixing at the time of sampling. Unfortunately, slowly increasing respiratory insufficiency prevented keeping the fish out of water for longer sampling periods.

The blood volumes shown in Table II are appreciably higher than typical estimates for teleosts (Table I). In part this is because Evans blue is known to be biased toward large estimates in mammals (Murray, 1964), probably because some dye escapes through capillary membranes before complexing with plasma proteins. This tendency is substantiated for salmonids because smaller estimates were obtained (Table II, test 4b) by reinjecting Evans blue into a second fish after all of the "loose" dye had been removed by the donor fish. Further, using hematocrit values in the calculations also tends to prejudice the estimate toward higher values, since the hematocrit is higher in major vessels than in the organs (Murray, 1964). Nevertheless, it still appears that the blood volumes of salmonids more closely resemble those of elasmobranchs (6.6 vol %) or agnathans (8.5 vol %) (Thorson, 1961) than those of most other teleost fish.

The primary reason for using Evans blue in spite of its recognized deficiencies was for making direct comparisons with earlier data, much of which were obtained with the Evans blue-hematocrit method. Conte et al. (1963) gave $3.5 \pm 0.9\%$ of body weight as the Evans blue blood volume of steelhead trout in fresh water, only slightly more than half of the present figure for the same species in sea water. The reinjected Evans blue (in coho) gave a smaller figure than standard Evans blue, somewhat comparable to that of Conte et al. when using Cr^{51} — labelled RBC — both being about 75% of the respective Evans blue figure. Thus, it is believed that the larger volume estimates obtained in this study are the result of improved sampling techniques rather than differences in the behavior of the dye.

In the present experiments, exsanguination was not intended to provide realistic estimates of blood volume. However, present data suggest the presence of larger amounts of blood than those to be expected from accepted blood-volume estimates. The data of Ronald et al. (1964) (Table I), indicate that about 80% of the total blood can be obtained by careful exsanguination of all major vessels. My data (Table II, test 5 and 8) suggest that approximately 50% of the blood volume as measured by reinjected Evans blue is readily

obtainable through a cannula in the dorsal aorta without emptying any other blood vessels. Therefore, if one accepts that salmonids have only about 3 ml blood/100 g body weight (the average of Conte's two radioisotopic methods most comparable to the reinjected Evans blue method), then one must also accept that over 75% of it can be withdrawn readily through a dorsal aortic cannula.

There is an opportunity with the present data to speculate about blood volume changes accompanying changes in environment. If the relationship between exsanguination and reinjected Evans blue (Table II, test 5 and 4b) also holds for the freshwater rainbow trout for which only exsanguination data are available (test 8), the blood volume of the latter would be about 6.[illegible]% of body weight as estimated by Evans blue. However, the Evans blue estim[illegible] for steelhead trout raised in sea water is 6.9% (test 7), suggesting that blood volumes may be larger in sea water, rather than smaller as suggested by the changes in freezing point depression which occur in the blood of salmon as they leave or re-enter fresh water (Benditt et al., 1941; Kubo, 1953; Parry, 1961). Since the seawater exsanguination data are based on only four fish, the present speculations only suggest the need for more comprehensive experiments.

In conclusion, the interpretation of Korzhuyev and Nikolskaya (1951) — apparently the only other persons who have found relatively large blood volumes in some teleost fish — seems applicable. They hypothesized that more active fish had larger blood volumes. Since salmon and trout can certainly be classed as active fish, the low blood-volume estimates obtained in salmonids by other investigators may be attributed to the fish's adverse reactions to handling which are accompanied by depressed circulatory activity (Randall et al., 1965).

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NOTES

Design of Small Holding Tank for Fish

Experimental studies employing juvenile Pacific salmon at this Station earlier necessitated some attention being directed toward the design of small holding tanks. Experience and evolution of ideas resulted in the construction of plastic tanks of a design described here.

Tank design has been based on three criteria: (1) the provision of circulation to maintain cultured fish in an active state; (2) the avoidance of projections, shapes, or surfaces which tend to cause injury; and (3) the provision of easily cleaned nontoxic surfaces which also would withstand continuous immersion without early failure.

Related to the foregoing considerations are the following observations. Young salmon held and forced to swim in flowing water have a higher capacity for activity than similar fish held under conditions of little or no water circulation. Circulating tanks can be made self-cleaning to minimize the accumulation of solid wastes. Rectangular tanks are more economic of space than circular tanks, yet circulation in the former produces "dead spots" in which debris will accumulate. Corners and rough surfaces are a source of injury to cultured fish, physical damage resulting from impact with the former and abrasion from the latter. Finally, early failure of many painted or otherwise prepared wood or metal surfaces resulted in the ultimate adoption of plastic as the fabricating material.

The tank design finally adopted was basically that of a rectangle with rounded corners, an inward slope to the sides and a central drain (Fig. 1, 2). Construction is of glass cloth and polyester resin, tanks being built up on an inexpensive plywood mold by the manufacturer.[1] The mould is constructed to provide a very smooth finish to the tank interior. The first resin coat on the mold is a colour coat which forms the inner surface of the tank. Glass cloth and subsequent resin coats are built up to a thickness of about $\frac{1}{8}$ inch. The resulting tank is strong enough to hold its shape without distortion when filled with water.

Two types of drain connection will be described. In earlier models a 4-inch diameter piece of $\frac{3}{4}$-inch plywood was bonded to the outside bottom of the tank at the drain position and overlaid with glass cloth and resin. This boss was drilled, prior to bonding, and subsequently tapped to receive a 2-inch nominal pipe size plastic close nipple. The resulting connection proved very strong but somewhat larger than might be desirable. Drain connections of

[1]Hallcraft Plastics Ltd., Vancouver, B. C.

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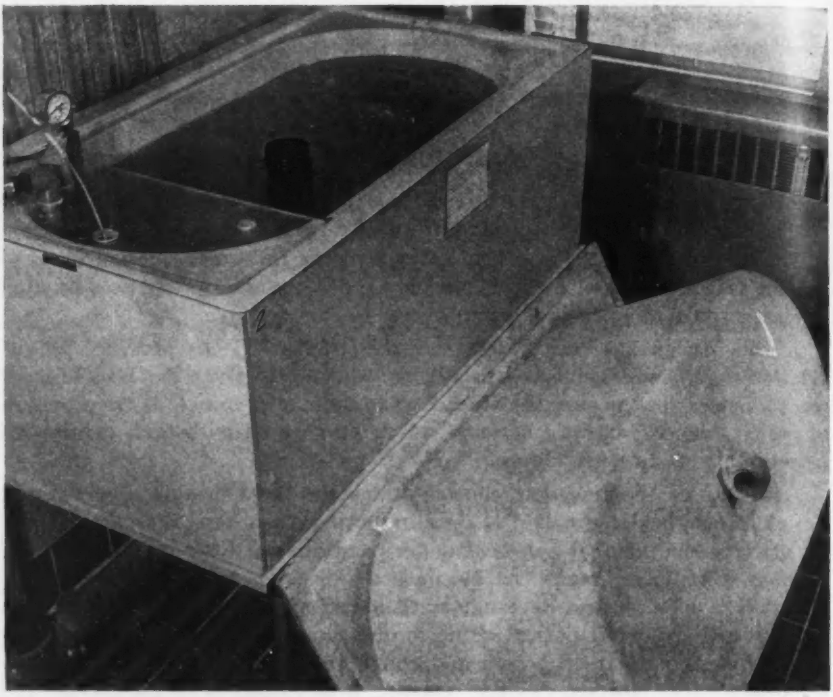
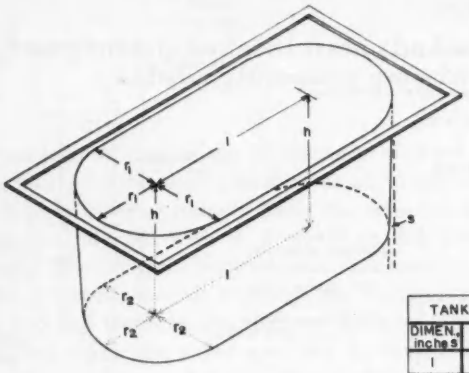


FIG. 1. A complete tank assembly is illustrated on the left, showing the plastic tank in a plywood box. The inlet pipe is held in position by a fixed piece of sheet acrylate (left). The concentric drain assembly is in the centre of the tank. A second tank (right) is shown upside down to illustrate the form of the tank and the drain connection.

later models were made more simply by setting a 1½-inch n.p.s. plastic elbow on the mold in the drain position and laying up the tank around it. In this case the elbow and point of contact with the bottom of the tank are reinforced with additional layers of glass cloth and resin. Details of these drain connections are illustrated in Fig. 3.

Two concentric pipes form the central drain. The inner waste pipe of 1½-inch n.p.s. plastic pipe is fitted to the drain connection according to the types of drain fitting employed (Fig. 3). If a 2-inch n.p.s. close nipple is used at the drain connection, 1½-inch n.p.s. plastic is used for the waste pipe and is either selected for size or turned on a lathe to provide a "slip-fit" inside the nipple. If a 1½-inch n.p.s. plastic elbow is used for the drain connection, the waste pipe is threaded for direct attachment to the drain elbow. In this latter case the waste pipe is threaded to a diameter slightly smaller than that of a standard 1½-inch pipe thread to ensure sufficient engagement for a good connection. The waste pipe is cut to a suitable length extending to within 1½–3½-inches of the lip of the tank. The outer concentric pipe is 3-inch n.p.s. rigid



TANK SIZE, (litres)			
DIMEN. inches	80	197	780
l	$7\frac{1}{2}$	24	24
h	$13\frac{3}{4}$	$16\frac{1}{2}$	36
r_1	9	10	15
r_2	8	9	13
s	1:h	1:h	2:h

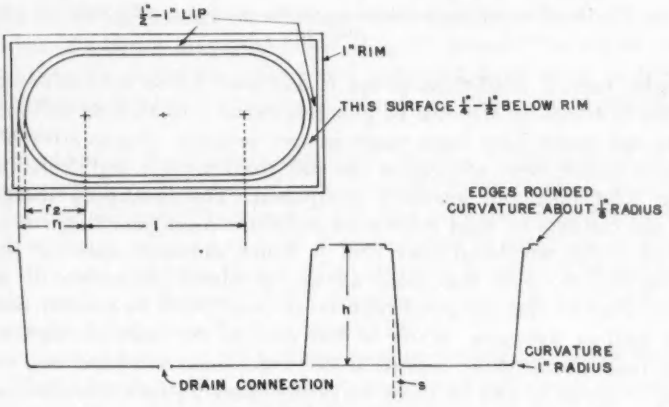


FIG. 2. Tank dimensions.

thin-walled plastic pipe[2] cut to a length to extend to within ½-inch of the lip of the tank. The bottom of this pipe is serrated or slotted to allow drainage from the bottom of the tank to enter the area between the concentric pipes thence up and out the waste pipe. The size of the slots is governed by the size of fish held in the tank. The central drain position results in virtually all waste materials being carried to the waste pipe overflow. The outer concentric pipe is held in position by plastic spacers (¼-inch sheet acrylate) cemented into grooves cut into the outer surface of the waste pipe (Fig. 3).

[2]Styrene acrylonitrile butadiene copolymer. All other pipe and fittings used are of unplasticized polyvinyl chloride. Both types of pipe are standard commercial products.

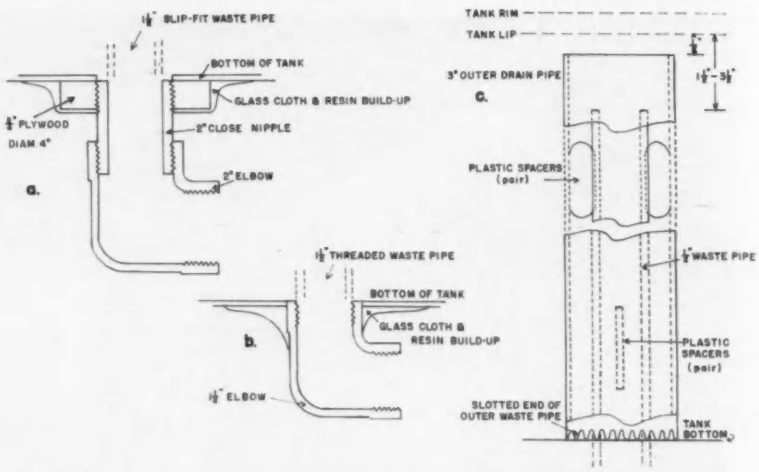


FIG. 3. Detail of two types of drain connection employed (a,b). Detail of central drain (c).

Under normal conditions of use it has been found convenient to set up the tanks in boxes constructed of ½-inch plywood mounted on suitable stands. Tops to the tanks have been made in two sections. One-quarter of the top consists of ¼-inch sheet acrylate at one end, fixed in place and drilled to receive a water inlet pipe and auxillary equipment. The remaining three-quarters of the top consists of rigid reinforced polyester resin panelling cut to fit the area within the peripheral tank rim. A 3-inch diameter hole cut in the top panelling directly over the drain allows for visual inspection of the waste overflow. Part of the top panel may be painted black to provide some cover for fish held in the tank. Water is delivered to the tank through a vertical piece of rigid plastic pipe, capped at the lower end, and fixed in a peripheral position. A series of two or three vertically spaced ⅛-inch diameter or smaller holes drilled in this pipe serve as horizontal jets, creating a circular current in the tank.

In storage the tanks nest because of the slight pitch to the sides. Three sizes of tank of the design described have been put to use, namely of 80, 197, and 780 litres (17.6, 43.3, and 171.8 Imperial gal) capacity. Many of these have been in constant use for over 5 years and have required no maintenance other than cleaning. The design adopted and materials selected for construction are regarded as having answered very satisfactorily the requirements for small holding tanks as originally envisaged.

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Microorganisms Isolated from the Hemolymph of the Lobster (*Homarus americanus*)

A survey to determine the incidence of *Gaffkya homari* among lobsters in the Canadian Atlantic area (Stewart et al., 1966) showed that 397 out of a total of 2035 lobsters examined, using the selective medium developed for the test, were not infected with *G. homari* but did have other bacteria in their hemolymph. The bacteria isolated with this medium do not necessarily reflect the actual predominance of types in the hemolymph, since the medium was selective and the test was qualitative. However, the genera isolated did vary with the area using the same medium. In area 4 (Stewart et al., 1966), for example, 79% of the isolates from false positive tests were *Micrococci*, while in area 5, 88% were rod-shaped bacteria. Their presence made confirmatory tests for *G. homari* necessary in the course of the survey.

This study was done because of the possibility that these bacteria might be pathogenic for the lobster and because their identification would aid in the development of a better diagnostic method for *G. homari*. The report describes the characteristics of the bacteria which appeared most often in the hemolymph of lobsters in area 5 and gives evidence of their lack of pathogenicity for the lobster.

Twenty-five of the lobsters examined in area 5 (Stewart et al., 1966) carried rods which produced acid from glucose. Seven of these isolates were selected for identification. Numbers 27, 78, and 86 were isolated from lobsters held captive in the pool for at least 3 weeks prior to sampling and numbers 132, 163, 223, and 250 were from freshly captured lobsters. The isolates were identified according to Bergey's (1957) classification using the methods outlined by the Society of American Bacteriologists (1957).

Description of isolates. All cultures were maintained in bacto[1] brain heart infusion (BHI) broth at 28 C and observations and tests were carried out on 24-hr cultures unless otherwise stated. On the basis of morphology and physiological characteristics they fell into the following groups:

GROUP A (Isolates 27, 86, 250). Rods (0.5 × 2.0μ), singly and in chains. Pleomorphic, forming coccoid cells on extended incubation. Gram-positive. Nonspore forming (Dorner method, Snyder modification, 4-day BHI agar slant). Motile (hanging drop) with peritrichous flagella (Bailey method, Fisher and Conn modification). Nonacid fast (Ziehl-Neelsen method). Facultative anaerobe (BHI agar shake culture). Good growth in BHI broth at 28 C; poor and no growth at 15 and 37 C, respectively. Good growth in BHI broth containing 3% NaCl; slight growth in 7% NaCl. Very slow growth in Bacto-Nutrient broth (Difco). Scant, transparent, beaded growth on agar stroke. Colonies convex and entire.

[1]Difco Laboratories Inc., Detroit, Mich.

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GROUP B (Isolates 78, 132, 223). Rods (0.5 × 1.5μ) occurring singly. Gram-negative. Motile with peritrichous flagella. No spores. Nonacid fast. Facultative anaerobe, showing very good growth at 25–28 C, scant growth at 15 C and good growth at 37 C. Good growth in BHI broth containing 3% NaCl; slight growth in 7%. Slow growth in Nutrient broth. Good filiform white growth on agar stroke. Colonies circular, convex, entire.

GROUP C (Isolate 163). Rods (0.5 × 1.5μ), occurring singly. Gram-negative. Motile with polar flagella. No spores. Nonacid fast. Strict aerobe. Very good growth at 28 C and good to no growth at 15 and 37 C, respectively. Good growth in BHI broth containing 3% NaCl, slight growth in 7%. Very good growth in nutrient broth. Good filiform growth on agar slope. Colonies circular, raised, entire. Water soluble brownish pigment after prolonged incubation.

The characteristics of group A isolates indicate that they belong to the genus *Brevibacterium*. Motility, nonliquefaction of gelatin, cultural characteristics (especially inability to grow well on nutrient agar), and beta-hemolytic property would suggest that they are *Brevibacterium incertum*.

Group B bacteria exhibit characteristics which might place them in the genus *Paracolobactrum*. However, on the basis of their lactose fermentation and nonreduction of nitrate, we have identified them as *Achromobacter superficialis*.

The bacterium in group C was identified as belonging to the genus *Pseudomonas*, but does not, however, fit the description of any of the species listed under this genus in Bergey's manual.

The cultures of *Pseudomonas* sp. and *Brevibacterium incertum* grew slowly (faint growth in 24 hr) and *Achromobacter superficialis* very slowly (faint growth in 48 hr) in lobster serum incubated at 15 C. Washed cell suspensions of these isolates standardized to McFarland turbidity scale 2 in sterile artificial sea water were injected (0.2 ml) into the ventral abdominal sinus of lobsters (mean weight 315 g) held at 15 C. No microorganisms could be detected in gram-stained hemolymph smears made after 4 weeks duration and the animals remained quite vigorous.

Because the bacteria were present in an animal to be prepared ultimately as a food product and, due to their possible pathogenicity for the animal, the sensitivity of the isolates to a series of antibiotics was determined *in vitro* using the method of Bauer et al. (1960) (Table I).

While this is to our knowledge the first reported occurrence of *Micrococci*, *Pseudomonas*, *Achromobacter*, and *Brevibacterium* in the hemolymph of the lobster, it is not surprising, since they are commonly found in the marine environment (ZoBell, 1946). They may have gained access to the hemolymph through injury to the digestive tract or integument. They are able to grow slowly in the serum and although they interfere with the presumptive test for *G. homari* they appear to be nonpathogenic for the lobster. Other microorganisms commonly associated with the lobster are the chitinovorous bacteria of Hess (1937) and *G. homari* (Hitchner and Snieszko, 1947; Snieszko and Taylor, 1947).

TABLE I. Characteristics of bacteria isolated from the hemolymph of the American lobster.

Test	Method	Cultures						
		A			B			C
		27	86	250	78	132	223	163
Gelatin liquefaction	Frazier method, Smith mod.	–	–	–	–	–	–	+
Hemolysis	3% defibrinated sheep blood in blood agar base	+	+	+	+	+	+	–
Litmus milk-acid	Bacto[a] Litmus milk	+	+	+	+	+	+	–
Nitrate reduction	Bacto BHI broth	–	–	–	–	–	–	–
Indole	Bacto SIM	–	–	–	–	–	–	–
H_2S	Bacto SIM	–	–	–	–	–	–	–
Methyl red	Bacto MR-VP	–	–	–	+	+	+	–
Voges-Proskauer	Bacto MR-VP	–	–	–	–	–	–	–
Oxidase	Skerman (1959)	–	–	–	–	–	–	–
Catalase	Skerman (1959)	–	–	–	–	–	–	+
Urease	Bacto urease (5 days)	–	–	–	–	–	–	+
Citrate	Bacto citrate	–	–	–	–	–	–	+
Chitinase	Benton (1935)	–	–	–	–	–	–	–
Acid from	Bacto nutrient broth base							
Glucose	(15 days)	+	+	+	+	+	+	+
Fructose		+	+	+	+	+	+	–
Lactose		–	–	–	+	+	+	–
Sucrose		+	+	+	+	+	+	+
Raffinose		–	–	–	–	–	–	–
Inulin		+	+	+	+	+	+	+
Mannitol		+	+	+	+	+	+	–
Antibiotic sensitivity	Multiple Sensi-Disc[b], Bauer et al., (1960). Oxoid[c] sensitivity test agar.							
Penicillin	2, 10 units	S[d]	S	S	MR	MR	R	R
Aureomycin	5, 30 mcg	S	S	R	S	S	S	S
Oxacillin	1 mcg	R	R	R	R	R	R	R
Ampicillin	2, 10 mcg	S	S	S	MR	MR	R	R
Vancomycin	5, 30 mcg	S	S	S	S	S	S	R
Streptomycin	2, 10 mcg	R	R	R	R	R	R	S
Polymyxin B	50, 300 units	R	S	R	R	R	R	S
Terramycin	5, 30 mcg	S	S	R	S	S	S	S
Coly-Mycin	2, 10 mcg	MR	MR	R	R	R	R	S
Demethylchlor tetracycline	5, 30 mcg	S	S	S	S	S	S	S
Triple Sulfa	0.25 mg, 1.0 mg	R	R	R	R	R	R	S
Chloromycetin	5, 30 mcg	S	S	S	S	S	S	R
Neomycin	5, 30 mcg	R	S	R	S	S	MR	S

[a]Difco Laboratories Inc., Detroit, Michigan.
[b]Baltimore Biological Laboratory, Baltimore, Maryland.
[c]The Oxoid Division of Oxo Ltd., London, England.
[d]S — susceptible; MR — moderately resistant; R — resistant.

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Northern Range Extension Records for Two Rockfish Species (*Sebastodes caurinus* and *S. elongatus*)

During May–August 1961 the National Museum of Canada conducted a marine biological expedition in the coastal waters of southeast Alaska and Prince William Sound (Bousfield and McAllister, 1962). Among the rockfish (Scorpaenidae) collected were two species not previously recorded from the area of capture.

COPPER ROCKFISH *Sebastodes caurinus* (Richardson 1845)

On July 9, 1961, a rockfish specimen identified as *Sebastodes caurinus* was collected by D. E. McAllister in Day Harbor, Blying Sound, Alaska (60°00′N lat; 149°06′W long). The fish, 89 mm fork length (sex not determined), was caught in a gill net in 40 ft (12 m) of water, and is now in the collection of the National Museum of Canada (cat. no. NMC 61-124). Additional specimens (220–305 mm, fork length) were caught in Prince William Sound, east of Blying Sound. A review of the rockfishes in Norman J. Wilimovsky's Alaska collection, now deposited with the Institute of Fisheries at the University of British Columbia, yielded one specimen of *S. caurinus* collected west of Cape Spencer. This fish, 190 mm fork length (sex not determined), was collected by jig in Port Dick, Alaska (59°02–19′N lat; 151°01–20′W long) (exact location not given), in 36 ft (11 m) of water by Rae Baxter on July 27, 1956 (cat. no BC 65-706).

Sebastodes caurinus closely resembles *S. maliger* in morphometric ratios, meristic counts, and other body characteristics such as head spines and color of peritoneum (Phillips, 1957). It differs from *S. maliger* in possessing two dark bars on the operculum radially directed posteriorly from the orbit, and lacking orange-brown spots on the lower anterior portion of the body and the deeply incised dorsal fin membranes.

Meristic counts and morphometric ratios were obtained from all Alaska specimens and these did not differ appreciably from those reported by Phillips (1957) for California specimens.

Phillips (1957) and Clemens and Wilby (1961) report the northern range limit for the copper rockfish as southeast Alaska (Dixon Entrance–Cape Spencer). The known range of this species is thus documented to extend westward approximately 500 miles from Cape Spencer, Alaska.

GREENSTRIPED ROCKFISH *Sebastodes elongatus* (Ayres 1859)

On July 13, 1961, a rockfish specimen identified as *Sebastodes elongatus* was collected by D. E. McAllister from a beam trawl catch 2 miles southeast of Green Island, Montague Island, Alaska (60°11′N lat; 147°26′W long), in 192–312 ft (59–95 m) of water (cat. no. NMC 61–140). The fish was 102 mm long, fork length, and sex was not determinable. A review of the

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rockfish collection at the Institute of Fisheries, University of British Columbia, failed to yield any specimens collected north of the Strait of Georgia except three specimens (242–299 mm fork length) collected by the author on September 7, 1965, off Forrester Island, southeast Alaska (54°49′N lat; 134°00′W long) (cat. no. BC 65-788).

Sebastodes elongatus resembles *S. proriger* in possessing the nasal preocular, postocular, tympanic, and parietal spines, a dark peritoneum, and horizontal bands along the body. It differs in possessing green bands rather than red, and 28–33 rakers on the first gill arch rather than 38–43. Morphometric ratios and meristic counts were collected from the four Alaska specimens and were found to be comparable to those reported for California specimens by Phillips (1957). However, close comparison was precluded by the paucity of specimens from Alaska and California, and difference in size range. Of special note was one southeast Alaska specimen which possessed supraocular spines.

Phillips (1957) lists the northern range limit for the greenstripe rockfish as Vancouver Island, B.C., and Clemens and Wilby (1961) list Strait of Georgia. Alverson et al. (1964) report catches in the British Columbia–southeast Alaska area (Cape Flattery–Cape Spencer) but do not list specific catch locations. The known range of this species is thus documented to extend northward and westward approximately 1100 miles from Vancouver Island, British Columbia.

The author is grateful to Dr D. E. McAllister for the opportunity to examine the rockfish collection of the National Museum of Canada, and to Dr N. J. Wilimovsky for similar courtesies extended at the Institute of Fisheries of the University of British Columbia.

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Lepomis megalotis, the Longear Sunfish, in Western Ontario

Twenty-eight specimens of *Lepomis megalotis* (Rafinesque) were collected by the senior author on July 8 and 9, 1960, from Burditt Lake (48°55′N lat, 93°47′W long) in Senn and Menary townships of the Rainy River District, Ont. (Fig. 1, no. 1). Scott (1963) has reported the collection as being from Clearwater Lake (the local name of Burditt Lake). These specimens appear to represent the northernmost known population of the species and details of biology and morphometrics are therefore of special interest.

Nineteen males and nine females were taken in a minnow trap shortly before the spawning season. Some females had large, well-developed eggs, in others, the eggs were of divergent sizes; the testes of the males were not enlarged. Table I lists the counts and measurements made from specimens and X-rays. Only 23 specimens were available for X-rays, and of these, two males (8.7% of sample X-rayed) had vertebral compactions or fusions of the

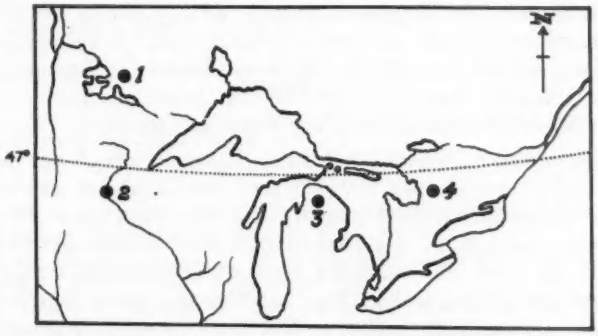

FIG. 1. A map of the Great Lakes Region showing the location of the present collection of *L. megalotis*, no. 1. Previous collections have been made south of the 47th parallel and are shown as no. 2, 3, and 4.

22nd, 23rd, and 24th centra, and therefore are not included in the vertebral count in Table I. The squamation, knob-like gill rakers, pointed pharyngeal teeth, and colour pattern agree with the descriptions of the species (Hubbs and Lagler, 1958; Jordan and Evermann, 1896; Trautman, 1957). One meristic character differs from the descriptions in the literature. Jordan and Evermann (1896) and Forbes and Richardson (1920) list 14 diagonal scale rows between the origin of the anal fin and the lateral line of *L. megalotis*. In the present collection, the number of scale rows below the lateral line varies from 11 to 14, with one specimen having 11, one having 14, and the remainder having 12 or 13 scale rows, with an overall mean of 12.2 (Table I).

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TABLE I. Counts and measurements made on specimens and X-rays of *L. megalotis*. Ranges are given in parentheses, and the means are above. Only 14 males and seven females are included in the vertebral count (see text).

Sex	No. fish	Total length	Fork length	No. of vertebrae (centra only)	Scales in lateral line	Dorsal rays	Anal rays
Male	19	63.5 (51–88)	60.9 (49–84)	29.2 (29–30)	5/35.9/12.2 (5/33–37/11–14)	X,11.2 (X,10–12)	III,10 (III,10–11)
Female	9	65.4 (57–77)	62.5 (54–74)	29 (29)	5/35.2/12.3 (5/33–37/11–13)	X,11 (X–XI,10–11)	III,10.1 (III,10–12)
Total:	28	64.8 (51–88)	61.9 (48–84)	29.3 (29–30)	5/35.5/12.2 (5/33–37/11–14)	X,11.1 (X–XI,10–12)	III,10 (III,10–12)

The specimens are probably *L. m. peltastes* Cope, 1870 (see Trautman, 1957). The population from which the specimens were taken is apparently geographically isolated from other populations of *L. megalotis*. Eddy and Surber (1943, 1960) cite the limit of northern distribution of *L. m. peltastes* as near Minneapolis in Minnesota (Fig. 1, no. 2). Hubbs (1945) disagreed, and with reference to Eddy and Surber's figure said, "It appears to represent one of the rare hybrid combinations, *Lepomis humilis* × *Lepomis macrochirus macrochirus*." Neither Evermann and Latimer (1910) nor Allin (1964) mention *L. megalotis* in their lists of fishes of northwestern Ontario.

Lepomis megalotis peltastes has been found in the lower peninsula of Michigan (Hubbs and Cooper, 1935) (Fig. 1, no. 3), and in southern Ontario as far north as Georgian Bay (Dymond, 1947; Radforth, 1944) (Fig. 1, no. 4). No records of stocking *L. megalotis* in northern Ontario were found. Until the present collection, the northern limit of distribution would appear to closely follow the 47th parallel (Fig. 1). The east–west distribution of the subspecies is from southern Quebec to Michigan (possibly Minnesota) (Hubbs and Lagler, 1958) and in the south from New York to Illinois and Iowa (Trautman, 1957).

A possible explanation for the occurrence of *L. megalotis* in the Lake of the Woods region is as follows. During the Pleistocene glacial period, a water connection existed between southern Minnesota and Lake Agassiz (which included the present Lake of the Woods region), through the Warren River to the Minnesota River and thence to the Mississippi River (Radforth, 1944). As the ice sheet retreated, Lake Agassiz drained to the north. The level of the lake became lower than the height of land across the northern part of Minnesota, severing the Warren River connection between the Mississippi and Lake of the Woods drainages. *Lepomis megalotis peltastes* is found in the Mississippi drainage and could have moved north through the Warren River into the present Lake of the Woods drainage, where they would be isolated as Lake Agassiz lowered. Underhill (1957) points out that, "There is

good evidence of a modern temporary connection between the Upper Mississippi River and the Hudson Bay basin." However, as there is no confirmation of *L. megalotis* in Minnesota, it is doubtful if *L. megalotis* has recently moved into the Rainy River District. Evidence is also presented by Underhill that, during the Pleistocene, other species migrated across the present watershed barrier, both from the south to north and north to south.

No records of *L. m. peltastes* are found for that portion of Ontario north of lakes Huron and Superior. Hence, a geographical isolation of *L. megalotis* seems to exist in the Lake of the Woods region.

Twenty-four specimens of the present collection have been deposited in the Royal Ontario Museum (R.O.M. cat. no. 21643), two are in the National Museum of Canada (not yet catalogued) and two are in the University of Ottawa collection.

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Combined Effect of Starvation and Severe Exercise on Glycogen Metabolism of Rainbow Trout, *Salmo gairdneri*

Miller (1958) demonstrated that glycogen reserves of hatchery-raised rainbow trout (*Salmo gairdneri*) were exhausted rapidly following transportation and planting in streams. Exhaustion of these stores was caused by increased activity. The aggressive resident trout population forced the introduced hatchery-raised trout into swifter running water where the food supply was less abundant. It is standard hatchery practice to starve fish 1 or 2 days prior to liberation to reduce water contamination by faecal material and regurgitated food. However, starvation in itself reduces energy reserves, particularly muscle and liver glycogen (Hayashi et al., 1964). The present experiment was carried out to determine the combined influence of starvation and exercise upon the glycogen reserves of 1.5-year-old rainbow trout.

Fish were 1.5-year-old fall-spawning Oregon domestic stock. These fish were hatched and reared at the Summerland Trout Hatchery, Summerland, B.C. The trout were transferred from an outside holding raceway to an indoor hatchery trough for study. The control fish were fed twice daily on Clark's Fish Feed. The ration was calculated at 0.9% body weight per day. Another 25 fish were starved for 84 hr prior to sampling. The control and starved fish were subdivided into two groups: those sampled without exercise and those sampled following 2 min vigorous exercise which was carried out in the manner described by Black (1955). Water temperature ranged from 10.5 to 11.5 C. Muscle and liver samples were taken from stunned fish and were quick-frozen (Black et al., 1962). Muscle lactate was determined on trichloroacetic acid filtrates by the method of Barker and Summerson (1941). Glycogen was determined on KOH digests by the anthrone method of Carroll et al. (1956) after precipitation in 60% ethanol (Connor et al., 1964).

Results are summarized in Table I. Muscle glycogen fell from 0.167% in the fed unexercised fish to 0.136% in the starved unexercised fish. Muscle glycogen fell to one-eighth of the resting value for fed fish exercised for 2 min, whereas in the starved-fish experiments muscle glycogen fell from 0.136 to 0.013% or to one-tenth of the starved unexercised value.

Muscle lactate changes for fed fish were similar to those reported by Black et al. (1962). The starved levels in the respective conditions overlapped those for fed fish. Liver glycogen values did not change significantly during exercise for either fed or starved fish ($p > 0.05$). The level of liver glycogen in the starved unexercised fish fell to 38% of the control value obtained for fed fish. From the foregoing data, it is clear that 84 hr of starvation caused a significant drop in liver glycogen ($p < 0.05$).

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TABLE I. Body weights, muscle and liver glycogens, and muscle lactates of fed and starved rainbow trout.

		Fed unexercised	Fed, exercised (2 min)	Starved unexercised	Starved exercised (2 min)
Body	No. of fish	12	12	12	11
weight	Average ± SE	262.5 ± 13.201	260.5 ± 13.655	252.5 ± 11.128	257.546 ± 23.98
(g)	Range	182–359	197–334	198–318	136–426
Muscle	No. of fish	12	11	11	11
glycogen	Average ± SE	0.167 ± 0.013	0.021 ± 0.004	0.136 ± 0.012	0.013 ± 0.003
(%)	Range	0.108–0.258	0.006–0.047	0.073–0.183	0.003–0.38
Muscle	No. of fish	11	12	11	12
lactate	Average ± SE	125.523 ± 13.292	434.838 ± 17.502	134.346 ± 14.740	415.432 ± 17.917
(*mg*/100g)	Range	47.11–184.22	331.31–549.55	58.97–227.33	318.08–504.85
Liver	No. of fish	12	12	12	11
glycogen	Average ± SE	3.661 ± 0.461	3.506 ± 0.587	1.375 ± 0.170	1.084 ± 0.142
(%)	Range	0.927–6.124	0.582–6.824	0.697–2.493	0.384–1.854

It is concluded that the present practice of starving hatchery-raised fish prior to transportation and liberation may place the fish at a disadvantage, particularly if liberated into a stream already containing an established population of fish. It is recommended that the method of starving fish before transportation and liberation be validated in the field.

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Tolerance Levels of Cod (*Gadus morhua*) to Low Salinity

Live cod (*Gadus morhua* Linnaeus) used at the Grande-Rivière Station were caught in the Bay of Chaleur where the salinity of the water ranges from 2.6 to 2.9% NaCl at the surface (Tiphane and Boudreault, 1963). A well near the shoreline by the Station supplies cold, sand-filtered water with a salinity of 1.8–2.2%. It was considered desirable to supply the Station's fish tanks with the well water, provided that the cod could tolerate the low salinity. Although considerable data are available concerning the effects of various levels of salinity upon fish (Black, 1957), no data specific for cod were found. This note describes an attempt to determine the tolerance levels of cod to low salinity.

The experimental tank consisted of a 700-gal, fiberglass tank, 6 ft in diameter. Water was continuously recirculated from the tank, through a heat exchanger, and back into the tank. The heat exchanger maintained the water temperature at 5–6 C throughout the experiment. The tank was initially filled with water containing 2.48% NaCl. Fresh water at a temperature of 11 C was added at various intervals to lower the salinity in a stepwise fashion. The temperature, *p*H, and salinity of the tank were recorded regularly. The *p*H varied from 6.9 to 7.3. Salinity was determined by the titration of the chloride ion with silver nitrate, using dichlorofluorescein as the indicator (Dyer, 1943). Figure 1 shows the record of the tank salinity during the course of the experiment.

The fish used in the experiment were caught by trawl line or gill net. They were held for 2 weeks in a recirculating seawater tank (salinity 2.53%, temperature 11 C) before they were placed in the experimental tank. This adapted them to captivity and weeded out weak individuals which had been injured during catching.

At the beginning of the experiment 13 cod were placed in the experimental tank. After 33 days the salinity was 1.33% and a second group of four cod was placed in the tank, directly from the seawater holding tank (2.53% salinity). At 65 days the salinity was 0.70% and a third group of 10 cod was placed in the tank, again directly from the holding tank. The sudden drop in salinity for the third group, from 2.53 to 0.70%, appeared to have no adverse effects upon the fish. The first fatalities occurred on the 69th day and the 82nd day, when four fish of the initial group died. Their death at this time was attributed to a failure in the air supply to the tank.

As the salinity in the tank was decreased, the fish became less active, and rested on the bottom more frequently than the fish in the seawater holding tanks. They showed an agitated behaviour after the 90th day when the salinity reached 0.46%. They surfaced frequently and were extremely sensitive to outside disturbances. They gradually became weaker until all were dead on the 111 th day.

As shown in Fig. 1, the first deaths occurred when the salinity was reduced to 0.27%. On further reduction of the salinity to 0.23% the remainder of the

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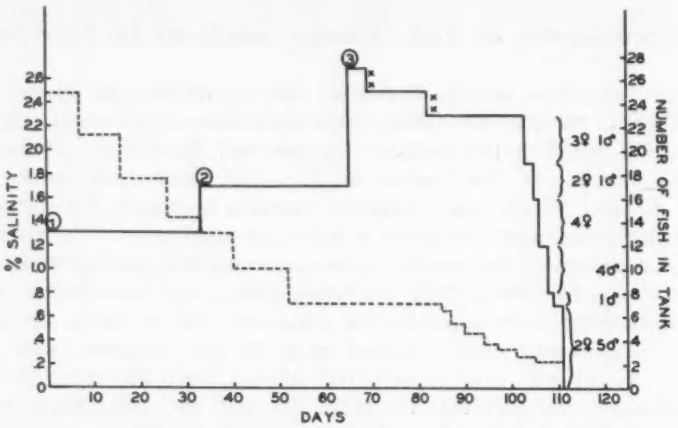

FIG. 1. Record of tank salinity in percentage NaCl and fish mortality. (1) Thirteen fish placed in tank; (2) Four fish placed in tank; (3) Ten fish placed in tank; X Fish which died when air supply to tank accidently cut off. ♂, ♀ Male and female fish which died when salinity in tanks reached lower levels.

fish died over a 6-day period. The length, weight, and sex of each fish were then determined. The females seemed more susceptible to the effects of low salinity and died first. Most of the fish were 58–62 cm long and weighed 1700–1800 g. However, among the large and small fish in the group size did not appear to be a factor in contributing to either an early or late death brought about by the low salinity.

It should be noted that the fish were not fed for the duration of the experiment. Starvation should not have contributed to mortalities since cod have been kept in seawater tanks at the same temperature and without food for periods up to 6 months.

It may be concluded that cod can safely tolerate water of low salinity such as the well water available at the Station. Other factors, such as *p*H, temperature, and dissolved oxygen are apt to be more important to the survival of cod held in aquaria. It also suggests that some inland aquaria holding marine specimens in artificial seawater might lower expenses by lowering the salinity of their tanks. Further studies could profitably be undertaken to determine the effect of temperature upon the tolerance levels of cod to low salinities.

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Northern Range Extensions for Three Species of Rockfish (*Sebastodes flavidus*, *S. paucispinis*, and *S. pinniger*) in the North Pacific Ocean

During August–September 1965 the Fisheries Research Board's research vessel *G. B. Reed* was used to conduct a survey of Pacific ocean perch (*Sebastodes alutus*) stocks from Cape Spencer, Alaska, to Cape Blanco, Oregon. Three species of rockfish (Scorpaenidae) not previously recorded from southeast Alaska waters were noted by the author. All were caught with a 350-mesh, semiballoon, groundfish trawl.

YELLOWTAIL ROCKFISH *Sebastodes flavidus* (Ayres, 1862)

On September 7, 1965, two rockfish identified as *Sebastodes flavidus* were collected 16 miles west of Forrester Island (59°49′N lat; 134°00′W long) in 102–106 fath (187–194 m). Both fish were females (397 and 430 mm, fork length). A review of the rockfish in Norman J. Wilimovsky's Alaska collections, now deposited with the Institute of Fisheries at the University of British Columbia, yielded a number of specimens from southeast Alaska. The most northerly collection was six specimens (255–283 mm, fork length) taken from a salmon trap (U.S. Fish and Wildlife Service No. 183) on the west shore of the Mansfield Peninsula, 2 miles north of Clear Point Light, Admiralty Island (58°16′25″N lat; 134°56′35″W long) on July 30, 1957, by J. M. Bally (cat. no. BC62-604).

Sebastodes flavidus resembles the dusky rockfish (*S. ciliatus*) and the black rockfish (*S. melanops*). It is readily distinguished from the former by its white peritoneum, and from the latter by the vertical or slightly posterior slant to the posterior profile of the anal fin (Phillips, 1957).

Meristic counts and selected morphometric ratios obtained from the eight Alaska specimens did not differ from those reported by Phillips (1957) for California specimens.

Clemens and Wilby (1961) list the northern range limit for the yellowtail rockfish as Hecate Strait. Alverson et al. (1964) report catches in the British Columbia–southeast Alaska (Cape Flattery–Cape Spencer) area, but do not give specific catch locations. The known range of this species is thus documented to extend northward approximately 250 miles from Hecate Strait.

The two *G. B. Reed* specimens have been deposited in the museum of the Institute of Fisheries, University of British Columbia (cat. no. BC65-788).

BOCACCIO *Sebastodes paucispinis* (Ayres, 1854)

On September 1, 1965, a rockfish identified as *Sebastodes paucispinis* was collected 11 miles west of Kruzof Island (57°14′N lat; 136°13′W long) in 114–120 fath (209–220 m). The fish was a male, 545 mm fork length. Sub-

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sequently, an additional six specimens (532–595 mm fork length) were collected south of this point to Dixon Entrance. A review of the rockfish collection at the Institute of Fisheries, University of British Columbia, yielded a number of specimens from southeast Alaska waters, but none were caught as far north as 57°N lat.

Sebastodes paucispinis resembles *S. brevispinis* in possessing a large, undershot jaw and few spines on top of the head. It differs in color and certain meristic counts (Phillips, 1957). Color of *S. paucispinis* is olive-brown to olive-red, and meristic counts are: gill rakers, 28–31; lateral line pores, 54–58; and diagonal scale rows below lateral line, 72–90. For *S. brevispinis*, color is olive-green to silver-grey, and the corresponding meristic counts are: gill rakers, 33–36; lateral line pores, 44–49; and diagonal scale rows, 58–70. Morphometric ratios and meristic counts for the seven *G. B. Reed* specimens fell within the ranges reported for California specimens by Phillips (1957).

Phillips (1957) and Clemens and Wilby (1961) list the northern range limit for bocaccio as Queen Charlotte Sound. Alverson et al. (1964) report catches in the Gulf of Alaska area (Cape Spencer–western Kodiak Island), but do not give specific locations. The known range of this species is thus documented to extend northward approximately 300 miles from Queen Charlotte Sound.

The seven *G. B. Reed* specimens have been deposited in the museum of the Institue of Fisheries, University of British Columbia (cat. no. BC65-787, 791, 792, 793, and 795).

CANARY ROCKFISH *Sebastodes pinniger* (Jordan and Gilbert, 1880)

On September 6, 1965, a rockfish identified as *Sebastodes pinniger* was collected 17 miles west of Cape Bartolome (55°16′N lat; 134°07′W long) in 94–100 fath (172–183 m). No other specimens were caught north of Dixon Entrance during the cruise, and none from Alaska waters was recorded in the rockfish collection of the Institute of Fisheries, University of British Columbia.

Sebastodes pinniger resembles *S. miniatus* in body shape, color of peritoneum, and spines on top of head. It is readily distinguished from this species by color (fresh specimens), gill raker count, and number of spines on the lower margin of the suborbital bone (Phillips, 1957). *Sebastodes pinniger* is grey, heavily mottled with orange and yellow, whereas *S. miniatus* is dark red, mottled with grey. Rakers on the first gill arch total 41–45 for *S. pinnige* , and 35–42 for *S. miniatus*. On the lower margin of the suborbital bone, *S. pinniger* has one triangular spine and *S. miniatus*, two. Some meristic counts and morphometric ratios of the *G. B. Reed* specimen differed from those reported by Phillips (1957) for California specimens. The more important values are as follows (California data in parentheses): anal fin rays, 8(7); lateral line pores, 45(39–43); anal fin base length into head length, 3.0(2.5–2.7); snout length into head length, 4.4(3.8–4.1). In addition the second anal fin spine was as long as the third whereas Phillips (1957) reports that for California specimens, the second anal fin spine is shorter than the third.

Phillips (1957) and Clemens and Wilby (1961) list the northern range limit for the canary rockfish as Dixon Entrance. Alverson et al. (1964) report catches from the British Columbia–southeast Alaska (Cape Flattery–Cape Spencer) and Shelikof Strait (central Alaska) areas, but do not give specific

locations. The known range of this species is thus documented to extend northward approximately 50 miles from Dixon Entrance.

The *G. B. Reed* specimen has been deposited in the museum of the Institute of Fisheries, University of British Columbia (cat. no. BC65-791).

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